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INDUSTRIAL ORGANIC ANALYSIS

FOR THE USE OF TECHNICAL AND ANALYTICAL
CHEMISTS AND STUDENTS

BY

PAUL S. ARUP, B.Sc., F.I.C.

Chief Chemist to English Margarine Works (1919) Ltd.

SECOND EDITION
REVISED AND ENLARGED

With Twenty-five Illustrations

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PREFACE

THIS work was written primarily for the use of students who might desire to gain some insight into the methods and principles of industrial organic analysis. As the first edition appears to have met a need, the present edition has been prepared, the opportunity having been taken to revise the text and to make additions which include a chapter on sugars, descriptions of certain recently published methods, and references to the literature of the subject-matter.

A selection has been made of such sections of the subject as appear to be of the greatest general interest and which may best be presented with the above-mentioned object in view.

For the most part this volume deals with naturally occurring substances or their immediate derivatives, the investigation of which possesses a fascination of its own. Such work, in which analysis is the primary method of attack, affords ample scope for research, valuable both from the scientific and the utilitarian points of view, while educationally it presents the advantage of affording illustrations of the application of theoretical knowledge to practical problems. The proper understanding of the processes described presupposes a thorough training in Chemistry and Physics, and the object has not been to suggest the acquisition of purely utilitarian knowledge at the expense of the valuable features of our college curricula.

Several points may be noted in connection with the work described. First, the importance of working with fair average samples wherever possible. (See, for example, pp. 8 and 41.) Second, the fact that many of the processes are of a semi-empirical nature renders the attention to experimental detail especially important; as the constitution of the substances dealt with is often complex and imperfectly understood, unsuspected sources of error may abound. Third, the cultivation of a sense of proportion should be aimed at; thus it will be useful to estimate how far errors in the weighing or measuring of the sample will be reflected in the accuracy of the results. On the one hand, waste of time through needless accuracy may be avoided, and, on the other, it may be found desirable to pay special attention, for example, to temperature corrections in the measurement of liquids, to the calibration of measuring vessels or thermometers. In actual practice, "short cuts" to the desired information may sometimes be taken with advantage, but there are dangers in dwelling too much on this subject here. Mention may, however, be made of the extreme usefulness of microscopic examination either as a preliminary test or as a means of gaining information which other methods fail to reveal. Again, it will be seen that many of the methods are designed for dealing with particular problems, thus differing from those which are taught for the analysis of mixtures of purely arbitrary composition. Fourth, the interpretation of results should be made a special study; here, also, special considerations may play an important part. Attention has been given to this side of the question in the text.

In conclusion, I wish to express my indebtedness to the authors of the treatises and papers mentioned here, and to those firms who have kindly lent the blocks for some of the illustrations. To Messrs. Bolton & Revi I am indebted for Fig. 14, reproduced from their "Fatty Foods," as well as for many trustworthy data, especially in connection with oils and fats. I have still to thank Professor J. C. Irvine, F.R.S., for kindly help and advice given to me when preparing the first edition of this work.

PAUL S. ARUP.

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ABBREVIATIONS

Temperatures are given in degrees Centigrade unless otherwise stated.

J.C.S.—*Journal of the Chemical Society.*

J.S.C.I.—*Journal of the Society of Chemical Industry.*

J.I.E.C.—*Journal of Industrial and Engineering Chemistry.*

INDUSTRIAL ORGANIC ANALYSIS

CHAPTER I

COAL AND COKE

INTRODUCTORY

COAL, which is at present the most important of the sources of energy available for industrial purposes, has resulted from the mineralisation of the wood of prehistoric plants, which has remained buried in the crust of the earth, and undergone changes as the result of which it has more or less completely lost its organic nature. In availing ourselves of the latent energy stored up in the coal, by burning it in our grates and furnaces, we are, broadly speaking, reversing the processes by which the plant made use of the radiant energy of the sun in carrying out the endothermic changes necessary to convert water, carbonic acid and simple mineral substances into complex vegetable tissues. In view of the complex nature of the process of mineralisation of wood, which has resulted in the formation of coal, as well as the difference in the conditions under which it must have taken place in different localities, it is hardly surprising that we should meet with an almost endless variety of coals, passing by almost imperceptible gradations, from the comparatively soft brown coals, or lignites, which have still preserved the fibrous structure of the wood, to the hard, black, shiny anthracites, which show conchoidal fracture, and display but little evidence of their organic origin.

Composition and Classification of Coals.—The chief uses of coal are as follows: (1) steam raising; (2) household use; (3) metallurgical and other manufacturing operations, and for smiths' forges; (4) the production of coke, gas, tar and ammonia.

Coals are broadly classified according to their technical applications, which may be determined from the results of chemical and physical examination. The interpretation of the results of a chemical analysis of coal generally falls under two headings. First, there are to be considered such constituents as water, sulphur and mineral matter, which must be regarded as more or less adventitious impurities, and which dilute the coal, reduce its calorific value or, if present in considerable amounts, may render it unsuitable for certain purposes. Second, the results of an analysis enable one to classify the coal, and to determine approximately the uses for which it is best suited. The soundest method for the classification of coals according to their chemical composition has been shown to be that which is primarily based on the percentages of carbon and hydrogen calculated on the coal, less water, sulphur and ash; thus, if S = the sum of the percentages of the water, sulphur and ash found in the coal, and C = the percentage of carbon found in the coal, then the percentage of carbon on the coal, less water sulphur and ash, will be given by

$$C \frac{100}{100 - S}.$$

The reason for eliminating these constituents from the calculation is that they may vary considerably in amount in coals of essentially the same type, and thus affect the percentages of carbon and hydrogen, as determined on the coal itself, to such an extent that no true comparison can be made.

The accompanying table shows how coals are broadly classified into five main groups, on this basis. The lignites and the true anthracites are omitted from the scheme.

Apart from the content of more or less adventitious impurities, such as water, sulphur and ash, the main points which come into consideration in judging of the value of a coal for any particular purpose are as in table on page 4.

The amount of gas and other volatile combustible matter formed on heating; this determines the suitability of the coal for gas or tar manufacture, and the length of flame on combustion.[1] The semi-anthracitic and anthracitic coals, which burn either with a short flame or practically no flame at all (Groups 4 and 5 in the above table), are more difficult to kindle than the long flame coals, but give a more intense heat, and produce little or no smoke. In these groups are included the well-known Welsh steam coals which are so largely used with marine boilers. Their freedom from smoke also renders them suitable for drying and curing hops and malt, and for horticultural purposes. Generally speaking, there is a direct relationship between the percentage of hydrogen and the length of flame, which both decrease in passing from Group 2 to Group 5.

---

[1] The nature and amount of gas and tar obtained depend very largely on the distillation temperature. See Chapter II.

Group	Per cent. on coal less Sulphur and Ash.			Percentage yield of Coke.	Character of Coke.	Sp. gr. of Coal.	Calorific Value of Coal in Calories.
	Carbon.	Hydrogen.	Oxygen				
1	75—80	5·5—4·5	19·5—15	50—60	Pulverulent or slightly sintered.	1·25	8,000—8,500
2	80—85	5·8—5·0	14·2—10	60—68	Intumesced; semi-fused.	1·28—1·30	8,500—8,800
3	84—89	5·5—5·0	11—5·5	68—74	Coherent and fairly dense.	1·30	8,800—9,300
4	88—91	5·5—4·5	6·5—5·5	74—82	Coherent and very dense.	1·30—1·35	9,300—9,600
5	90—93	4·5—4·0	5·5—3·0	82—90	Pulverulent or slightly sintered.	1·33—1·40	9,000—9,500

Some coals (Groups 2, 3 and 4) become fused or semi-fused on heating or burning; these are referred to as caking coals. The non-caking coals, which are included in Groups 1 and 5, do not soften in this way, so that the individual lumps remain separate during combustion and provide for a freer access of air than do the caking coals. These peculiarities, as well as the relative length of the flame, must be taken into account in considering the type of furnace for which the coal is best suited. The caking power also influences the nature of the coke; thus, it will be noticed that the non-caking coals of Groups 1 and 5 produce pulverulent cokes, while those of Groups 2, 3 and 4 all produce more or less coherent cokes. The condition of the coke is further affected by the amount of gas formed on heating or combustion, which, generally speaking, varies directly with the percentage of hydrogen. We accordingly find that in passing from Group 2 to Group 4, the coke becomes denser and less porous.

The non-caking coals of Group 1 differ from those of Group 5 in burning with a long flame. They are mainly used for steam raising and are also suitable for gas making in some cases.

The coals belonging to Groups 2 and 3 are sometimes referred to as the "bituminous coals," though it should be understood that they have no connection with the substance known as bitumen. The softer varieties, which are rich in volatile matter, are included in Group 2, and are usually known as the cannel coals; they are generally used for making gas, tar and ammonia. The coals of Group 3, which yield more coke than those of the former group, are used in smiths' forges and for manufacturing coke. Gas, tar and ammonia are sometimes also obtained from these coals.

The true anthracites and the lignites, which have not been dealt with hitherto, take their places at either end of the scale in the above classification. The former contain, as a rule, over 93 per cent. of carbon, and less hydrogen than the anthracitic coals belonging to Group 5; they burn without flame, are shiny jet-black in appearance, show conchoidal fracture, and sometimes have a specific gravity as high as 1·6. The lignites, on the other hand, contain less carbon and more hydrogen than the coals of Group 1; they are brownish-black in appearance, comparatively soft, and show a fibrous structure; owing to the relatively large amount of wate and mineral matter which they contain, they have a lower calorific value than the true coals. Commercially they are of considerably less importance than the latter.

For further information on this subject, see the Monograph on the Constitution of Coal mentioned at the end of this chapter; the monograph contains a very complete list of references, and a review of the chief systems of classification of coals.

The relations between the chemical composition of coals and their chief properties and uses will be treated of in greater detail when the methods for determining the various constituents are described.

The above classification, and the explanatory remarks which follow it, must not be interpreted in too literal a sense; it is very difficult to place all coals in clearly defined groups forming a simple system of classification, while their uses, though primarily dependent on their chemical composition, must, in many cases, largely be determined by commercial considerations, such as the cost of the coal itself, cost of transport, and the value of the products obtainable from it (*i.e.* gas, tar, ammonia or coke), which latter can only be satisfactorily determined by trials on a manufacturing scale. It is, however, generally possible to form an idea of the uses to which a coal may be put with greatest advantage from the results of a chemical examination; and moreover, if a certain kind of coal has been found to be suitable for a certain purpose, chemical examination will show whether subsequent supplies are likely to fulfil the same conditions as the original sample. Again, it will always be useful to have a check on the water, ash and sulphur, which, if present in large amounts, invariably reduce the value of the coal as fuel. The price paid for coal for industrial purposes should always be based on the results of analysis; this is not always done in the United Kingdom, but the practice is fairly prevalent in America. The official procedure[1] is to multiply the contract price by the actual calorific value found, and to divide it by the value guaranteed by the vendor; a certain addition to or deduction from the figure thus calculated is made according to the percentage of ash, the scale being steeply graduated with increasing percentages of ash. It should always be borne in mind that no analysis of coal will be of much value unless carried out on a fair average sample. (See below under "Sampling.")

The table on pages 12 and 13 shows the results of analyses, collected from various sources (most from "Analyses of British Coals and Cokes"), of typical coals and cokes, and their principal uses; together with the classification and explanatory remarks given above, it may be used as a guide for interpreting the results obtained by the analytical methods described below.

---

[1] U.S. Geological Survey, Bulletin No 428.

CHEMICAL EXAMINATION OF COAL AND COKE

Sampling.—In all technical and commercial analytical work, the operation of taking the sample for analysis is of the utmost importance; in the case of coal and coke, where such large masses are dealt with, this remark applies with especial force; it is obvious that a carefully picked sample may differ very considerably in composition from a sample which has been taken systematically and without prejudice, with the object of ascertaining, as far as possible, the average composition of the entire mass. Although the sampling of coal and coke can hardly be described as a laboratory operation, directions for systematic sampling are given here, owing to the extreme importance of this operation to the analyst. Whether or not the student has had the opportunity of taking his sample from a large mass of material, he should, at all events, follow out the latter part of the following directions in dealing with the reduced sample.

Quantities of coal or coke, as the case may be, amounting in all to 2 to 3 cwt., are taken at equal intervals during unloading,[1] or from all sides as well as the interior of the heaps, and removed to a place where the material will be protected from rain, dampness or direct sunlight. The whole is broken up on a smooth floor into pieces about the size of apples, well mixed and arranged in the form of a square layer about 20 cm. deep. Diagonals are marked out on this square, and the material in two opposite triangles is removed; the remainder is then rearranged into a square, and the process repeated until about 200 lbs. remain, which are broken into pieces about the size of nuts, and systematically reduced, by the process just described, to about 10 lbs. The sample thus obtained is preserved in air-tight metal boxes till required for use, when it is further reduced as follows:—The whole is well mixed, whereupon 500 grams are abstracted in such a way as to obtain an average sample, and reduced to a fine powder, preferably in a small hand mill, so constructed as to avoid loss of dust during the grinding. A. C. Fieldner[1] recommends the use of a porcelain ball mill for the final grinding; if a suitable grinding surface is not used, the ash content of the coal may be affected by the abraded matter. A. E. Findley[2] recommends breaking the coal into a coarse powder between linen; once broken down, it does not undergo increase in ash content on grinding in an iron or ordinary mortar. E. G. Bailey[3] has worked out mathematically the sizes to which coal and coke should be broken up in order that the error in the determination of the ash may be less than one per cent., the object being to obtain a sample which shall contain slate and coal in representative proportions. The powder is placed in a dry bottle provided with a well-fitting glass stopper, and thoroughly mixed by shaking. Samples for analysis are taken from this bottle as required.

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[1] The subject of sampling of coal deliveries is treated of by G. S. Pope, *U.S. Bureau of Mines*, Bull. 63.

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[1] "Accuracy and Limitations of Coal Analysis," *Chemical Engineer*, 17, p. 50.

[2] *J.S.C.I.*, 1919, 38, p. 93 T.

[3] "Accuracy in Sampling Coal," *J.I.E.C.*, 1909, 1, p. 101

Hygroscopic Water. (*a*) *In Coal.*—Two grams of the powdered coal are weighed between a pair of well-ground watch glasses, so that moisture shall not be attracted through undue exposure to the air, and dried at a temperature of 105° on the shelf of an air oven, till constant in weight; the time required is usually about two hours. Overheating of the sample must be avoided, or appreciable quantities of volatile matter other than water may be lost; the process is always liable to a certain error due to the cause just mentioned, as well as oxidation of the coal, which occurs, to a slight extent, when the latter is heated in contact with air to the temperature employed for expelling the water. In most cases, however, the results thus obtained will be found to be sufficiently accurate for industrial purposes.

Fieldner (footnote 1, p. 9), discussing the accuracy of moisture determinations in coal, states that agreement to within 0·15 per cent. between duplicate analyses may be obtained if the ¼-inch mesh sample is air dried at 30° to 40° and then reduced to 60 mesh in a ball mill; he dries one gram of the sample for one hour at 105°.

If the sample is subsequently to be analysed for hydrogen, the moisture should be determined very accurately, for errors here will affect the figure for hydrogen. An accurate method is as follows:—2 grams of powdered coal are spread in a thin layer on a clock glass and placed over sulphuric acid in a vacuum desiccator which is then evacuated. The sample is weighed every 24 hours till no further loss in weight takes place; before and during the weighing, the coal should be covered by a clock glass and exposed to the air as little as possible. If a good vacuum is maintained in the desiccator, the drying should be complete in 24 to 4 hours. The Committee on Coal Analysis of the American Society for testing materials and the American Chemical Society, in their preliminary report,[1] recommend that a vacuum of 3 mm. be maintained in this process. The Committee recommend other accurate methods involving drying in currents of dry inert gases, preferably nitrogen; these methods, however, require special apparatus.

Last, it may be mentioned that if the coal contains clay, the latter must be got rid of if the sample is to be submitted to elementary analysis, for some clays do not lose all their water below 700°. A. Lissner[2] recommends treating the coal with a mixture of two parts of commercial hydrofluoric acid and one part of concentrated hydrochloric acid, after which it may be dehydrated completely.

The proportion of hygroscopic water present in coals is very variable; excluding the lignites, which may contain up to 30 per cent., coals, especially when freshly raised, may contain as much as 15 per cent. of water, though this limit is seldom exceeded. Broadly speaking, a good coal should not contain more than 3 to 4 per cent. of water, while in a good anthracite the percentage of water should lie between *nil* and 1·5, or at most, 2.

Moisture. (*b*) *In Coke.*—According to Arth, 100 to 200 grams of coke, broken into small lumps, are weighed into a porcelain dish and dried in an air oven at 150 to 160°, until constant in weight. Samples should be kept in air-tight vessels, as they easily lose their water on exposure to air.

Moisture not only dilutes the fuel, but also reduces its calorific value owing to the heat which it absorbs on

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[1] *J.I.E.C.*, 1913, 5, p. 517. [2] Abs. *Analyst*, 1910, p. 133.

Description of Coal or Coke.	Carbon, per cent.	Hydrogen, per cent.	Oxygen, per cent.	Nitrogen, per cent.	Sulphur, per cent.	Ash, per cent.	Water, per cent.	Volatile Combustible Matter, excluding water, per cent.	Coke, less ash, per cent.	Percentage Composition, excluding Sulphur, Ash and Water. Carbon.	Percentage Composition, excluding Sulphur, Ash and Water. Hydrogen.
Anthracite, Pennsylvania .	90·5	2·4	2·5	*Nil*	*Nil*	4·7	*Nil*	—	—	94·9	2·5
Anthracite, S. Wales . .	90·4	3·3	3·0	0·8	0·9	1·0	2·0	—	—	93·5	3·4
Welsh steam coal (T. Hughes) .	87·7	4·0	2·2	1·0	1·0	2·8	1·3	8·0[1]	87·0	9·24	4·3
Steam, manufacturing and house coal, Leicester . .	74·9	4·5	15·0	1·2	0·9	3·0	13·2	30·0	54·2	90·4	6
Coking coal, Durham (J. Pattinson) . . .	83·84	4·7	3·8	1·3	0·8	4·2	1·5	22·7	71·8	89·5	5·1
Steam, manufacturing, coking and house coal, Lancashire .	81·7	5·0	5·8	0·9	0·5	2·9	2·4	20·3	77·9	86·6	5·3
Gas coal, Durham . . .	84·5	5·2	3·9	1·6	1·3	2·3	1·2	29·1	67·4	88·7	5·4
Gas coal, Durham (J. Pattinson)	82·4	5·1	6·6	1·1	0·9	2·0	2·0	31·3	64·7	86·6	5·4
Steam coal, Durham . .	81·2	5·2	6·2	—	0·9	2·2	2·1	31·9	63·8	86·3	5·5
Gas coal, Commentry, France (Mahler)	8·02	5·3	7·2	1·0	—	3·4	3·0	34·4[2]	—	85·7[2]	5·6[2]

Steam coal, Blauzy, France (Mahler)	79·4	5·0	8·7	1·1	—	1·9	3·9	31·9[2]	—	84·6[3]	5·3[3]
House gas and manufacturing coal, Derbyshire . . .	68·0	4·5	10·2	1·2	0·6	3·8	11·8	32·7	59·7	81·1	5·3
Steam coal, Staffordshire .	78·6	5·3	12·9	1·8	0·4	1·0	1·1	—	—	79·7	5·4
Cannel coal for gas and tar, Boghead	65·3	9·1	5·4	0·7	0·1	18·6	0·5	—	—	80·8	11·2
Lignite (Bohemian) . .	63·94	5·05	21·70	0·83	0·45	6·22	1·81				
Peat (Bornstein) . . .	43·76	4·16	24·57	2·30	0·24	7·94	17·03				
Malting coke, Alloa, Scotland (J. W. Napier) . . .	94·8	—	—	—	0·5	4·7	0·1				
Coke (R. Tatlock and Thomson)	90·4	—	—	—	1·2	7·1	0·3	1·0			
Coke (McCreath) . . .	89·6[4]	—	—	—	0·8	9·1	0·03	0·5			
Coke, Connelsville, U.S.A. .	87·5[4]	—	—	—	0·7	11·3	0·5	0·01			

---

[1] Volatile matter, excluding water and sulphur.

[2] Volatile matter calculated on coal, less ash and water.

[3] Carbon and hydrogen calculated on coal, less ash and water.

[4] Non-volatile carbon.

evaporation. It is sometimes the custom to specify a certain limit for the amount of hygroscopic water in coal, and to make a proportionate deduction in the price paid if this limit is exceeded.

Ash.—The residue of mineral matter left on complete combustion of the coal or coke is weighed as ash. The amount of ash thus found does not represent the total amount of mineral matter originally present in the fuel, and, moreover, varies somewhat with the mode of combustion, depending, among other things, on the amount of sulphur left in the residue and the amount of carbon dioxide lost by the alkaline earth carbonates. Fieldner (footnote 1, p. 9) states that the difference between igniting at a low red and a bright red heat may lead to a difference of one per cent. in the ash. The determination of the ash should be carried out separately, and not combined with the elementary analysis or the determination of volatile combustible matter and coke.

The following directions for the determination are due to Arth:—1 to 5 grams of the finely powdered coal (or coke) are weighed off in a platinum dish or, failing this, a porcelain dish, about 7 cm. in diameter; at first a gentle heat is applied, in order to avoid caking and subsequent difficulty in combustion, the dish being covered with a piece of platinum foil so long as there is any danger of loss by decrepitation. The subsequent heating is best carried out in a muffle furnace, the dish being placed on a piece of platinum foil, to avoid contact with the siliceous material of the furnace. The temperature is gradually raised to a bright red heat; if the layer of ash is thick it should be cautiously stirred from time to time with a stout piece of platinum wire. When the combustion is judged to be complete, the dish and its contents are cooled in a desiccator and weighed. Complete combustion is effected with difficulty if graphitic matter is present in the residue; unburnt carbon should be tested for in the cooled residue by adding a few drops of alcohol, when the carbon particles will, if present, be observed floating on the liquid. In this case the alcohol should be removed by evaporation and the combustion of the residue completed over the blow-pipe flame. In difficult cases, the combustion may also be facilitated by allowing a gentle current of oxygen to impinge on the residue while it is being heated, care, of course, being taken that particles of ash are not blown away.

Owing to the differences in results obtained on igniting at different temperatures, it is desirable that the process should be standardised as far as possible; the American Committee (footnote 1, p. 11) recommend that the temperature of the muffle should be kept at 700° to 750°.

The ash percentage is one of the most important figures obtained in the analysis of coal. The proportion of ash is greatest in coals which are rich in volatile combustible matter; in these it may sometimes exceed 15 or even 20 per cent. As in the case of the water, a certain maximum limit for the ash in coal may be specified by the purchaser, say 8 or 10 per cent, and a deduction made in the price paid if the limit were exceeded. The undesirability of a large proportion of ash in coal is obvious; the ash dilutes the fuel, tends to obstruct the draught during combustion, and carries with it some of the heat of the fire, as well as unburnt fuel, on falling from the grate. Generally speaking, it may be said that a good coal should yield less than 6 per cent. of ash. The same applies to coke, especially when used for metallurgical or similar operations, in which the chemical action of the ash has to be considered.

Volatile Combustible Matter, Combustible Residue and Coke.—When coal is heated out of contact with air, part of the carbon is lost, mainly in the form of volatile hydrocarbons and carbon monoxide; hydrogen, oxygen, water and ammonia are given off at the same time. The non-volatile residue, which consists of an impure carbon containing the greater part of the mineral matter of the original coal, is known as coke. The total volatile matter, less the moisture, is determined as "volatile combustible matter"; the non-volatile residue, less the ash, is known as the "non-volatile combustible residue," the "fixed combustible residue," or the "fixed carbon." The latter term should be avoided, as it is somewhat misleading.

The importance of the yield and nature of the coke has already been pointed out; the yield and nature of the volatile combustible matter are of importance if the coal is to be used for gas or tar manufacture, and the yield of volatile combustible matter, as determined by a laboratory operation, is a useful guide in classifying the coal under examination. Bearing in mind that the amount as well as the chemical composition of the volatile matter obtained from a coal vary with the conditions under which the destructive distillation is carried out,[1] it follows, first, that the results of any laboratory operation should not be interpreted too literally in judging of the probable behaviour of a coal when submitted to destructive distillation on a manufacturing scale, especially as regards the nature of the distillate, and, second, that the estimation of the volatile and non-volatile matter should be carried out, as far as possible, under certain standard conditions. The following methods are recommended by the American Committee (footnote 1, p. 11) :—

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[1] See Chapter II., p. 38. A high distillation temperature results in the production of a hard coke, a low temperature in the production of a soft coke.

One gram of the fresh, undried coal is heated for exactly seven minutes in either of the following ways :—

(*a*) In a 10 c.c. crucible stood on a tripod in a muffle at 950°, or (*b*) in a 20 c.c. crucible set 1 cm. above the top of a No. 4 Meker burner, which has an outside diameter at the top of approximately 25 mm., the flame being not less than 15 cm. high, the temperature being controlled at 900° to 950° either by a thermo couple or by the melting point of potassium chromate (940°). The crucibles should be covered by well-fitting lids. The crucible heated in the open should be protected from draughts. The upper surface of the crucible lid should burn clear, but the under surface should remain covered with carbon. The volatile combustible matter, determined by loss in weight, should be calculated on the dry coal. Duplicate analyses should agree within 0·5 per cent. Fieldner (footnote 1, p. 9) recommends a temperature of 950° to 1000° for all coals ; he states that anthracites show bad agreement between duplicate analyses if the temperature limits are not adhered to closely, but that softer coals show better agreement with varying temperatures.

The coke and non-volatile combustible residue may be estimated from the weight of the residue in the crucible, the latter by deducting the ash as previously determined.

R. Lessing[1] has constructed an electric furnace consisting of a silica tube wound with platinum wire and fitted with an inner silica tube in which one gram of the sample is placed; it is claimed that the use of this apparatus enables the swelling properties of coal to be seen very clearly, and that the differences in behaviour of various coals on coking can be better studied than by the usual crucible method.

The accompanying table shows the percentages of volatile matter yielded by the different varieties of coal, anthracite and lignite, as determined by Mahler:—

Description of Coal.	Volatile combustible matter calculated on coal, less ash. Per cent.
Anthracites	3 to 5
Semi-anthracitic . .	6 to 14
Bituminous for steam and coking	15 to 25
Bituminous for blast furnaces and forges . .	25 to 30
Cannel	About 50
Lignites of good quality .	About 50

The outstanding feature to be noticed here is the gradual diminution in the volatile matter in passing from the cannels to the anthracites. The above groups, from the cannels to the semi-anthracitic coals, inclusive, roughly correspond with the Groups 1 to 5, respectively, in the table on p. 4.

The association of a high percentage of volatile matter with a high percentage of ash is sometimes explained by the assumption that the mineral matter has enclosed the mother substance of the coal, and thus hindered its decomposition by the escape of gas.

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[1] *J.S.C.I.*, 1912, 31, p. 465.

Coke produced at high temperatures will only contain slight quantities of volatile matter. The softer cokes produced at low temperatures usually contain about 7 to 11 per cent. of volatile matter; the products of the "Coalite," "Tarless Fuel," and the "del Monte" processes may be regarded as intermediate between the typical coals and cokes; they should find increasing use in the future as substitutes for coal on grounds of fuel economy, as the distillates are valuable as motor fuel.[1]

Coke should, in view of its method of production, only contain slight quantities of volatile matter. If desired, this may be determined as described for coal.

Sulphur.—This constituent is usually present in coal in the form of iron pyrites and calcium sulphate. When the coal is submitted to destructive distillation part of the sulphur is driven off, chiefly in the form of organic sulphur compounds, owing to the decomposition of the pyrites. The determination of sulphur in coal and coke is generally carried out by the Eschka-Fresenius method, as follows:—

1 gram of the finely powdered material is mixed in a platinum crucible with twice its bulk of a mixture of 1 part of dry sodium carbonate and 2 parts of calcined magnesia; the uncovered crucible is placed in an inclined position, and its lower half is heated to a low red heat till the colour of the contents changes from a grey to a yellow or brown; it is then allowed to cool; the ash mixed with one half to one gram of ammonium nitrate,

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[1] H. E. Armstrong, *J.S.C.I.*, 1916, 35, p. 220. Evans, *ibid.*, 1918, 37, 212 T.

and heated again to redness for 5 to 10 minutes, the crucible this time being covered with a lid. After cooling, the crucible is placed in a beaker of water, and the contents detached by heating the liquid. The crucible is removed, washed carefully with water in order to remove adhering solution, and the washings added to the contents of the beaker. During the process of ignition just described, the sulphur contained in the coal or coke will have been oxidised to soluble sulphates, which may be determined in the solution by precipitating with barium chloride and weighing the precipitate, in the usual manner described in all text-books on quantitative inorganic analysis.

The limits of variation of the proportion of sulphur in coal are usually from one half to 2 per cent., while in anthracites it may only be present in the smallest traces. Generally speaking, it may be said that a good coal or coke should contain less than 1 per cent. of sulphur. It is important that coke for use in the blast furnace should contain a minimum of this constituent owing to its undesirable influence on the properties of the iron.

Nitrogen.—This constituent exists in the coal in the combined form, and originates from the proteins present in the original wood. When coal is submitted to destructive distillation, the nitrogen escapes mainly in the form of ammonia, and also as aromatic amines, pyridine bases, etc. The nitrogen in coal is conveniently estimated by the method of Kjeldahl, which is very commonly used for the estimation of nitrogen in foodstuffs, fertilisers and other materials. The method depends on the destruction of nitrogenous matter resulting in the formation of ammonium sulphate, by the action of boiling concentrated sulphuric acid in the presence of mercury sulphate or other catalysts. When the digestion with the acid is complete, the mixture is made alkaline, and the ammonia distilled off into a known volume of standard acid and estimated by titration. Modifications of the Kjeldahl method are often used either for convenience' sake or in order to suit particular cases. Gunning's modification consists in the use of sulphuric acid and potassium sulphate, while in the Gunning copper method copper sulphate is added as a catalyst. The use of potassium sulphate ensures a higher digestion temperature than is reached with sulphuric acid alone; it has, however, been pointed out by P. A. W. Self[1] and by E. Carpeaux[2] that loss of ammonia occurs if the composition of the mixture approximates that of potassium hydrogen sulphate, which may occur if too much potassium sulphate is used or too much sulphuric acid is lost by evaporation. The method of digestion given below is that recommended by Fieldner and Taylor,[3] who have determined the conditions necessary for obtaining accurate results with coal.

1 gram of the powdered coal is introduced into a Kjeldahl digestion flask together with 30 c.c. of pure concentrated sulphuric acid, 15 grams of potassium sulphate, and a drop of mercury. The flask (see Fig. 1) should be made in resistant glass, and should have a capacity of about 500 c.c. Placing the charged flask in an inclined position on a piece of wire gauze, heat is carefully applied until all frothing has ceased; the mixture is boiled till colourless, and then cooled for ten

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[1] *Pharm. Journ.* 1912, 88, p. 384 (Abs. *Analyst*, 1912, 37, p. 203).

[2] *Ann. Chem. Anal.*, 1913, 18, 315 (Abs. *Analyst*, 1915, p. 172).

[3] *J.I.E.C.*, 1915, 7, p. 106.

minutes; powdered potassium permanganate is added in small quantities at a time till a green or purple colour remains after shaking, and the mixture is further boiled for one to one and a half hours. After cooling, water is added, small quantities at a time being allowed to flow

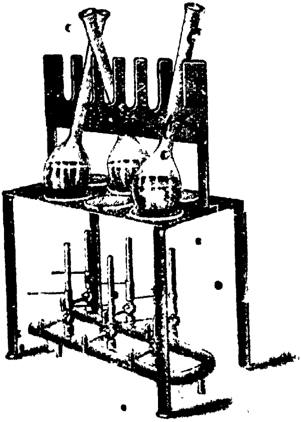

FIG. 1.- Kjeldahl Digestion Flasks on Stand.

down the side of the flask, while the contents are continually being agitated; the whole is transferred to a 700 c.c. flask, the total water added, including rinsings, amounting to 200 to 300 c.c. When quite cold, a few pieces of zinc foil are added to secure even boiling, and an excess of concentrated sodium hydroxide solution (about 90 c.c. of a 50 per cent. solution) is poured down the side of the flask in such a way as to avoid, as far as possible, its mixing with the acid liquid; sufficient of a 4 per cent. solution of potassium sulphide is added to precipitate all the mercury (usually about 25 c.c.), and

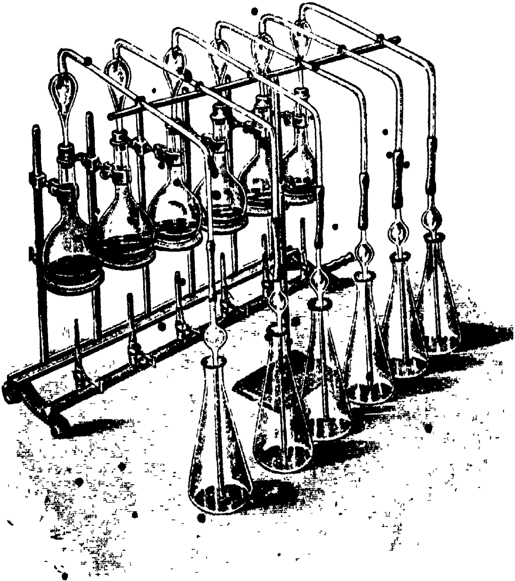

FIG. 2.—Distillation Apparatus for Kjeldahl's Process for the Determination of Nitrogen.

the flask is connected up without delay by means of a rubber stopper with the distillation apparatus.

The accompanying illustration (Fig. 2) will give an idea as to the general arrangement of the apparatus. The receiving flask contains a definite volume of decinormal sulphuric acid, and the tube, which dips just below the surface of the acid liquid, is provided with a bulb in order to prevent suck-back into the distillation flask. For this purpose an ordinary pipette may be used. If preferred, the vapour may be condensed in a straight tube condenser, in which case the condenser tube should be brought near the surface of the standard acid, not dipping into the latter. If only a few analyses are to be made, ordinary Liebig condensers, fitted with adapters, may be used; if many analyses are to be made, it is more convenient to employ a series of metal tubes placed vertically in a metal tank through which water may be made to circulate, as shown in Fig. 3. Arrangements such as this admit of a number of distillations being carried out simultaneously. Sometimes the distillation is carried out from the original digestion flask, avoiding transference. Whatever the arrangement used, the distillation flask should be connected with a bulb-trap for preventing alkaline spray from being carried over into the receiver. When 250 c.c. have been distilled, all the ammonia is certain to have been distilled over into the acid, which is then titrated with decinormal sodium hydroxide or baryta solution in order to determine the amount of ammonia absorbed. The indicator used may be litmus, methyl orange or cochineal, but not phenol phthalein.

It is necessary to perform a blank test in order to determine the amount of ammonia in the materials used, which should, however, be very small. If the analysis is carried out for the first time, a parallel determination should be carried out with a pure organic substance containing nitrogen, such as hippuric acid. The proportion of nitrogen in coal may vary from 0·2 to 2 per cent. The determination of this constituent is chiefly of interest as furnishing a rough estimate of ammonia and other nitrogenous products obtainable on distillation of the coal. If this question does not come into consideration, the estimation is often omitted, and the nitrogen is grouped together with the oxygen, in the statement of the results of the analysis, both being determined by difference.

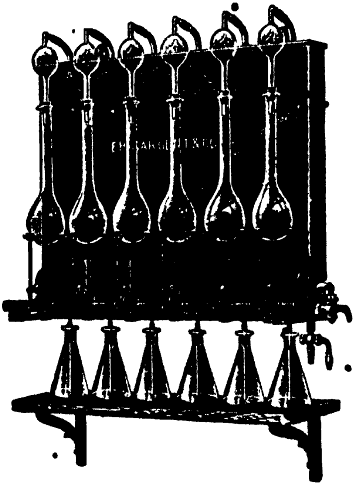

FIG. 3.—Distillation Apparatus for Kjeldahl's Process for the Determination of Nitrogen.

The Kjeldahl method and its modifications applied to other substances.—Many other substances do not require such drastic treatment in the digestion process as coal does. Thus, it is often sufficient to boil for half an hour after the liquid has become colourless, and to omit the boiling after the addition of the permanganate. In the Gunning modification it is usual to employ 25 c.c. of sulphuric acid and 12 grams of potassium sulphate; in the Gunning copper modification, 0·3 gram of copper sulphate is added to this mixture as a catalyst; it is no trouble and often safer to employ the latter modification in preference to the former. The use of copper sulphate has the advantage that the subsequent addition of potassium sulphide becomes unnecessary. Mercuric sulphate is, however, a more efficient catalyst than copper sulphate, and must be used with substances which are difficult to decompose completely; in the original Kjeldahl method, which is often used, the digestion mixture consists of 20 to 30 c.c. of sulphuric acid and 0·7 gram of mercuric oxide or the equivalent of mercury. In the Gunning Arnold method both copper and mercury are used, one gram of the sample being treated with 25 c.c. of sulphuric acid, 16 grams of potassium sulphate, one gram of copper sulphate, and one gram of mercuric oxide. It is advisable to use a definite amount of mercury or oxide, for a simple trial will then show how much sulphide solution need be added in order to have an excess beyond that required to precipitate all the mercury, and thus to decompose the mercurammonium compounds which do not give up ammonia on boiling in alkaline solution. For a similar reason the relative strengths of the sulphuric acid and the caustic soda should be determined.

O. F. Jensen[1] compared the results obtained by the Gunning Arnold method with those obtained by the Gunning copper method with bone meal, dried blood, cyanamide and linseed meal; he found that quantitative yields were obtained by either method, digesting for one to one and a half hours, though blood required three hours' digestion in the Gunning copper method. The Gunning Arnold method is used for pepper in which the nitrogen is mainly present as piperine, a heterocyclic compound of the pyridine type; B. Dyer[1] found that this method gave good results with pyridine.

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[1] *J.I.E.C.*, 1915, 7, p. 38.

Modified Methods to include Nitrogen as Nitrates.—Nitrogen present as nitrates will, of course, be lost in the processes described above, which, however, suffice for the analysis of the substances mentioned. The methods described under this heading are especially applicable to fertilisers which may contain considerable amounts of nitrates.

Gunning's Method.—0·5 to 3·5 grams of the sample are well mixed with 35 c.c. of pure sulphuric acid in which has been dissolved 3 per cent. of salicylic acid, and the whole is shaken at frequent intervals for 10 minutes. 5 grams of sodium thiosulphate and 10 grams of potassium sulphate are then added, and the mixture is carefully heated until frothing has ceased, after which it is further treated as directed above.

Iodlbauer's Method.—The digestion mixture consists of 20 c.c. of concentrated sulphuric acid, 2·5 grams of phenol sulphonic acid (made by dissolving 50 grams of phenol in sufficient sulphuric acid of specific gravity 1·84 to make 100 c.c.), 2 to 3 grams of zinc dust and five drops of chloroplatinic acid. The mixture is heated and should become colourless in four hours.

Carbon and Hydrogen.—The importance of knowing the percentages of carbon and hydrogen for the purpose of classifying the coal has already been pointed out in the introductory section of this chapter. The determination of the calorific value of the coal, as described below, is also based on the elementary analysis.

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[1] *Analyst*, 1895, 20, p. 252.

The method employed for the determination of carbon and hydrogen in coal is essentially the same as that employed for determining these constituents in organic compounds which contain nitrogen and sulphur. The details of the operation will be found described in many books dealing with practical organic chemistry, and will therefore not be repeated here; it will only be necessary to call attention to a few points of interest in the present case.

About half a gram of the finely powdered coal should be weighed off in a porcelain boat, about 7 cm. long and 7 to 8 mm. broad, and then mixed with about twice its bulk of dry, finely powdered copper oxide, great care being taken to avoid loss. The combustion tube should be packed throughout with lead chromate, in the usual way, in order that all sulphur may be retained as lead sulphate and prevented from escaping as sulphur dioxide, a short reduced copper spiral being placed in the rear end, in order to decompose nitrogen oxides. While volatile matter is being distilled from the coal, oxygen should only be allowed to pass through the tube at the rate of one bubble in two seconds; the speed may be doubled when only coke is left. The lead chromate should not be heated so strongly that it fuses with the glass of the tube; moreover, lead sulphate, which will be formed by the interaction of the chromate and sulphur of the coal, is not quite stable at such high temperatures.

In calculating the percentage of hydrogen from the water absorbed by the drying tube, allowance must be made for the hygroscopic water, accurately determined as described above, this being deducted from the total water weighed, in order to arrive at the water formed by combustion of the hydrogen. As has already been pointed out, powdered coal is hygroscopic, so that unless the sample for analysis is preserved in a well stoppered bottle during the interval between the water determination and the elementary analysis, serious errors may be made. The re-calculation of the percentages of carbon and hydrogen on the coal, less water, ash and sulphur, has been described on p. 2. The method for calculating the calorific value of the coal from the results of the elementary analysis is described below.

Oxygen.—There is no direct method available for determining this constituent; it is therefore determined by difference, deducting the sum of the percentages of the carbon, hydrogen, hygroscopic water, ash, sulphur and nitrogen from one hundred. The figure thus obtained will naturally be affected by the accumulated errors of the previous determinations; the oxygen content is, for this and other reasons, no longer used as a basis for the classification of coals. If the nitrogen has not been estimated, both this and the oxygen will be determined together, by difference.

The amount of hydrogen found in excess of that required to combine with the oxygen in the coal (excluding, of course, the oxygen of the hygroscopic water) to form water, is known as the "disposable hydrogen." Generally speaking, the disposable hydrogen may be taken as a relative measure of the amount of gas obtainable from the coal, and an indication of the length of flame which will be produced on combustion. These relationships will be apparent on referring to the tables given above.

Determination of the Calorific Value of Coal and Coke.—The amount of heat theoretically obtainable from a given weight of coal or coke determines to a great extent its value as a fuel; it is often the custom to base the commercial valuation of fuels on the results of determinations of calorific value, which have been carried out on fair average samples.

The calorific value of a fuel may be stated in several ways:—

(*a*) As the number of calories produced by burning 1 kilo. of the fuel, the calorie being the amount of heat required to raise the temperature of 1 kilo. of water from 4° to 5° C.

(*b*) As the number of British Thermal Units obtainable from 1 lb. of the fuel, the B.T.U. representing the amount of heat required to raise the temperature of 1 lb. of water through 1° F. at 39·1° F.

(*c*) As the number of pounds of water which may be evaporated at the boiling point by the combustion of 1 lb. of the fuel.

Determination of Calorific Value by Calorimeters.—Calorimetric methods are generally used in practice. Two instruments in common use are the Lewis Thompson and the "Sarco" Mahler-Donkin bomb calorimeter. The Lewis Thompson calorimeter consists essentially of a copper cylinder fitting on to a copper base by clips; the coal, mixed with potassium chlorate and nitrate, is burnt inside the cylinder, the gaseous products of combustion passing through holes near the base of the cylinder and up through the water in the calorimeter vessel. This instrument is very largely used for the commercial valuation of coals; it is much less expensive

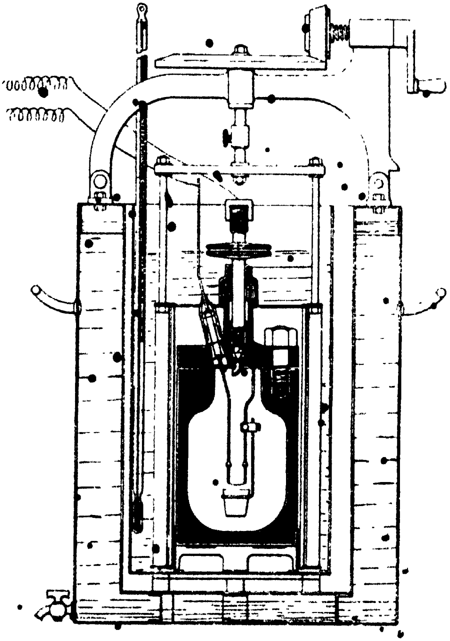

FIG. 4.—"Sarco" Mahler-Donkin Bomb Calorimeter.

than the bomb types, but does not give such accurate results, and is not suited for use with anthracitic coals containing more than 87 per cent. of coke, or with coke itself. The bomb calorimeters consist essentially of strong brass vessels which are immersed in outer calorimeter vessels containing water. The coal is fired electrically inside the bomb, which is charged with oxygen under pressure. In addition to accuracy, bomb calorimeters have the advantage that they can be used for liquid and gaseous fuels.

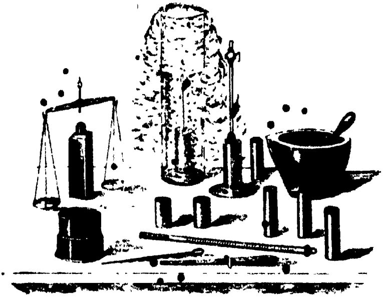

FIG. 5 Lewis Thompson Calorimeter.

Coals containing over 2 to 3 per cent. of moisture should be dried before burning in the calorimeter. In calculating the calorific value allowance should be made for the dilution of the fuel by the moisture when the dried coal is tested, and also for the latent heat of evaporation of the moisture, including that formed by the combustion of the hydrogen. It must be remembered that in the calorimeter the water is deposited in the liquid form, whereas in practice it escapes as steam. The accuracy of working may be checked by making a combustion with pure cane sugar, the calorific value of which is 3957·0 calories.

Detailed descriptions and directions for use are supplied by the makers, and may also be seen in dealers' catalogues; they will readily be followed by anyone acquainted with the principles and practice of calorimetry.

Calculation of Calorific Value from the Results of Elementary Analysis.—The calculation is based on Dulong's assumption that the amount of heat given out on the burning of a fuel is equal to the sum of the amounts of heat produced on the combustion of its separate elements, the whole of the oxygen present being considered as already combined with sufficient of the hydrogen present to form water. Knowing the percentages of the carbon, disposable hydrogen and sulphur present in the coal, and the calorific values of these elements, it should be possible to calculate the calorific value of the fuel itself. The above assumption is, of course, not theoretically justifiable, but experience has shown that when applied in the case of coal, the results obtained are sufficiently accurate for practical purposes. The present method cannot be applied to liquid fuels, as here the heats of formation of the constituent compounds are too considerable to be left out of account.

The following data are employed in the calculation:—

					Calories.
1 kilo. of	Carbon	in burning to	CO_2	produces	8,140
1 ,,	Sulphur	,,	SO_2	,,	2,160
1 ,,	Hydrogen	,,	H_2O as steam	,,	28,900

The latent heat of evaporation of water = 600

In practice, it is usual to take the calorific value of hydrogen as 34,500 calories per kilo. Then if

H be the percentage of Hydrogen,
C ,, ,, Carbon,
S ,, ,, Sulphur,
O ,, ,, Oxygen,
and W ,, ,, Hygroscopic water,

the quantity of heat, Q calories, obtainable from 1 kilo. of the fuel is usually calculated from Dulong's formula :—

$$Q = 81 . C + 290 \left(H - \frac{O}{8}\right) + 22 . S - 6 . W,$$

the water being liberated as steam.

Representative results of calorific determinations on the various kinds of coal are included in the table on p. 4. As a rule, the results obtained for coal by the method described do not differ from those obtained by the bomb calorimetric method by more than about 2 per cent.; in many cases the variation is less than 1 per cent. Mahler has shown that the variations in the results obtained by the two methods are considerable in the case of lignites, peat, and especially mineral oils. The calorific values of the latter fuels cannot be accurately calculated from the carbon and hydrogen content, but must be determined by the calorimetric method.

The heat actually available in practice naturally falls short of the amount of heat theoretically obtainable on combustion of the fuel; owing to loss of heat with the chimney gases, with the ash as it falls from the grate, radiation and incomplete combustion, the available heat is usually less than 80 per cent. of that theoretically obtainable. The results of laboratory determinations of calorific value are, however, none the less valuable, as they enable the analyst to estimate the relative heat values of different fuels or kinds of coal when used under approximately similar conditions.

PHYSICAL EXAMINATION OF COAL AND COKE.

In the table on p. 4 will be found the specific gravities of the different varieties of coal; it will be noticed that there is a gradual increase in the specific gravity in passing from the varieties which contain least carbon to the anthracitic group. The determination of this constant may usually be omitted, as surer indications of the nature of the coal are obtained from a chemical examination.

It is sometimes of importance to determine the porosity and the crushing strength of coke; the latter is of importance when the coke is to be used in the blast furnace, as it should be capable of bearing the weight of the material above it without being crushed to powder, in which case it would tend to obstruct the draught. As has been pointed out previously, the nature of a coke depends, not only on the nature of the coal from which it has been prepared, but also on the method of preparation; this point is discussed in Chapter II., p. 38. The methods for determining the porosity and crushing strength of coke are fully described in Stillmann's "Engineering Chemistry."

REFERENCES

"Engineering Chemistry," by Stillmann. 1910. Williams and Norgate, London, and the Chemical Publishing Co., Easton, Pa.

"Technical Methods of Chemical Analysis," Vol. I., Part I., by Lunge, translated by Keane. 1911. Gurney and Jackson, London.

"Contributions à l'Étude des Combustibles," by Mahler. 1903. Librairie Polytechnique, Paris.

"Chemisch-Technische Analyse," by Post. 1908. Fr. Vieweg und Sohn, Brunswick.

"Analyses of British Coals and Cokes," by Greenwell and Elsden. 1907. Chichester Press, London.

"Gas and Fuel Analysis for Engineers," by Gill. 1911. Wiley and Sons, New York.

"An Introduction to the Study of Fuel," by Brislee. 1912. Constable and Co., London.

"Fuel and Refractory Material," by Humboldt Sexton. 1909. Blackie and Son, London.

"Monograph on the Constitution of Coal," by Stopes and Wheeler. 1918. Published for Department of Scientific and Industrial Research by H.M. Stationery Office.

"Coal, Coke and Bye Products, Methods for Commercial Sampling and Analysis," by J. M. Camp. Carnegie Building, Pittsburgh, Pa., U.S.A. 1918.

"Coal, its Composition, Analysis, Utilisation and Valuation," by Sommermeier. 1912.

"Coal and its Scientific Uses," by W. H. Bone. 1918. Longmans, Green and Co.

"Standard Methods of Chemical Analysis," Part II., by W. W. Scott. 1917. Crosby Lockwood, London. (D. van Nostrand, New York.)

"Modern Coking Practice," Vol. I., by J. E. Christopher. 1917. Crosby Lockwood, London. (D. van Nostrand, New York.)

CHAPTER II

COAL TAR AND ITS DISTILLATION PRODUCTS

INTRODUCTORY

WHEN coal is submitted to dry distillation it yields three main volatile products, viz., coal gas, water containing ammonia in solution, and coal tar. The present chapter deals with the examination of the last-mentioned substance and its chief distillation products, with special reference to their application in the production of materials for the manufacture of dyes, disinfectants and other valuable substances, derived chiefly from the aromatic hydrocarbons. In this connection it should be mentioned that tars which are unsuitable for the above purposes have recently attained enhanced importance as sources of motor spirit (benzol) and fuel oil, the demand for which is likely to increase still further. (See below.) Coal tar is chiefly obtained as a by-product, either in the manufacture of coke, in which case it is known as coke-oven tar, or in the manufacture of coal gas, when it is known as gas tar. Blast furnace and generator gas tars, which are sometimes obtained as by-products in the manufacture of pig iron and producer gas, respectively, have not the same commercial importance as the two first mentioned products.

Properties and Composition of Coal Tar.—Crude coal tar is a dark brown or black, more or less viscous fluid, smelling of creosote. It is usually heavier than water, the tars having specific gravities under 1·000 being useless for the production of dyes. The latter point will be more fully dealt with below.

As pointed out in the previous chapter, the proportion of tar yielded by different kinds of coal varies considerably; the nature of the tar is influenced, not only by the quality of the coal from which it is distilled, but also to a considerable extent by the distillation temperature. Generally, when low temperatures are employed, the resulting tars consist mainly of liquid and solid paraffins, olefines and the more complex phenols. Higher temperatures, on the other hand, tend to give rise to the formation of aromatic hydrocarbons, free carbon, and phenol rather than homologues of phenol; olefines and acetylenes are formed in smaller quantities, while the paraffins practically disappear. The following example, given by Dr. F. Mollwo Perkin,[1] illustrates well the differences in nature and amount between the products of distillation at high and low temperatures:—

At High Temperature (about 900°). Per ton of coal, 12,000 cubic feet of gas, 20 lbs. of ammonium sulphate, 11 gallons of tar consisting mainly of aromatic hydrocarbons suitable for producing dyes, etc. Coke hard.

At Low Temperature (about 350° to 550°) Per ton of coal, 5000 cubic feet of gas, 10 lbs. of ammonium sulphate, 20 gallons of tar consisting mainly of aliphatic hydrocarbons, olefines, paraffins and also naphthenes, and unsuitable for producing dyes, etc.; tar acids (phenols) are also present, but these are only suitable for use as disinfectants and not for synthetic purposes. Coke soft.

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[1] Lecture given before the Institute of Petroleum Technologists, 17th Dec., 1918.

High distillation temperatures are essential for the production of gas, both as regards quantity and quality, and tars from which aromatic hydrocarbons and phenols, etc., may be conveniently separated in a state of sufficient purity for synthetic work; the presence of paraffins is highly objectionable in this connection. On the other hand, the value of low temperature distillation products has been recognised within the last few years. It will first be noted that the yield of tar is greater than at high temperatures; from rich cannel coals as much as 40 to 60 gallons may be obtained per ton; again, such tar has a lower specific gravity and yields a greater proportion of low-boiling constituents than high temperature tar. The benzol fraction forms a good substitute for motor petrol, the demand for which has increased enormously owing to the development of aircraft among other reasons. In this case the presence of paraffins is no objection. The higher boiling products are fuel oil, which, like the heavy petroleum products, is suitable for use in motors of the Diesel type, lubricating oil, and disinfectant phenols. Finally, paraffin wax may be obtained from this tar. The soft coke produced at low temperatures contains from 7 to 11 per cent. of volatile matter, and is well suited for domestic purposes; from what has been said, the wastefulness of the system of burning raw coal will readily be realised. (See footnote, p. 19.) Gas coke is very dense and graphitic owing to the high temperature employed. Coke sufficiently hard and dense for use in metallurgical operations is produced in coking ovens where somewhat lower temperatures are employed, the object being to make a coke which will stand the weight of the materials in the blast furnace. Compared with the gas retort, the coking oven yields slightly less gas and a tar not quite so rich in aromatic hydrocarbons, but which may, however, generally be used for making products for synthetic purposes. Lunge ("Coal Tar and Ammonia") points out that the action of heat tends to encourage molecular condensations with the formation of substances such as naphthalene and anthracene, and the elimination of hydrogen, either in the elementary state, or in the form of highly hydrogenated hydrocarbons such as methane. The tendency towards the formation of phenol rather than the cresols at higher temperatures is similarly explained, while the formation of free carbon, either in a finely divided state or as a graphitic mass, may be regarded as the last stage resulting from the tendency towards molecular condensation brought into play by the action of heat.

Coal tar, as produced for the manufacture of colours, contains the following substances as its most important constituents :—

Water and ammonia.

Benzene, toluene, xylenes and higher homologues of benzene.

Phenol, cresols, naphthols and other phenolic substances of higher molecular weight.

Amine bases such as aniline and its homologues.

Naphthalene, anthracene, phenanthrene and their homologues, as well as other hydrocarbons containing condensed benzene nuclei, of high molecular weight, such as pyrene and chrysene.

Pyridine, quinoline and bases of a similar nature.

Nitriles, carbazoles, etc.

Sulphur containing compounds, of which the chief are carbon disulphide and thiophen.

In order to isolate the chief constituents, the coal tar is submitted to a preliminary distillation in retorts placed over an open fire. The following fractions are collected:—

(1) First runnings, up to about 120° C.
(2) Light oils or crude naphtha, up to about 170° C.
(3) Middle or carbolic oils, from 170° to 230° C.
(4) Creosote oil, from 230° to 270° C.
(5) Anthracene oil, over 270° C.

The first two fractions are often collected together, in which case they are referred to as the "total light oils"; they are accompanied by the aqueous ammonia which remained in the crude tar after centrifuging (usually 2 to 3 per cent.). The still residue consists of pitch.

Molinari ("Organic Chemistry") gives the following mean compositions for gas and coke oven tars:—

	GAS TAR per cent.	COKE-OVEN TAR per cent
Benzol to 135°	36·12	12·66
Benzol 135°-165°	15·59	16·42
Phenol oils	18·01	18·47
Residual middle oils	26·51	49·36
Water and loss	3·67	3·09

Reference may be made to Butler's Article on "Modern Practices in Coal Tar Distillation,"[1] and "Notes on the Commercial Fractionation of Benzene, Toluene, and Xylenes."[1]

Sampling.—The importance of proper sampling was pointed out in dealing with coal (see p. 8). As the sampling of a viscous liquid like tar likewise requires careful attention, a few words may be said on the subject. The following recommendations are due to Weiss and others.[1] If the tar is being pumped, a half-inch sampling pipe is inserted in a line with the flow of the tar, half-way to the centre of the main pipe on the discharge side of the pump, the inner open end of the pipe being turned at an angle of 90° so as to face the flow of the tar. One gallon per 1000 gallons is thus taken off, kept at a temperature of not over 49°, and as soon as the pumping is over, the large sample is thoroughly mixed and a quart sample is taken from it. If the tar is dealt with by gravity, it may be continuously sampled by a small drip pipe inserted into the main pipe, or by dipper full samples taken at frequent and regular intervals. Tar in tanks is sampled at various depths by the use of small weighted bottles fitted with tight stoppers which may be pulled out with string, or by sampling cocks at different levels. In each case the large sample is mixed and dealt with as already described. The sample for analysis should be kept in a well-closed vessel.

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[1] Butler, *J.S.C.I.*, 1918, 37, 23 T. Butler and Popham, *ibid.*, 1918, 37, 220 T.

Specific Gravity.—The water is first separated as follows: the tar is allowed to stand in a conical flask immersed in water at 50°, for 24 hours; the water collecting at the top is poured off as completely as possible, the rest being absorbed by drawing a piece of filter paper over the surface.[1] The tar is then cooled to 15° and its specific gravity determined at this temperature. For this purpose, the pyknometer or hydrometer cannot well be used owing to the viscosity of the tar at ordinary temperatures. Lunge advises the use of an ordinary cylindrical weighing bottle of about 50 c.c. capacity, a vertical groove, about 2 mm. wide and 2 mm. deep, being cut in the glass stopper. The bottle is weighed dry and empty, and then, when filled with water at 15·5°, in the usual way. It is then about two-thirds filled with the tar and placed, without the stopper, in hot water for one hour to get rid of air bubbles. After cooling, the bottle and stopper are weighed with the tar. Boiled distilled water is then added to fill the bottle, and the whole weighed after allowing to stand in water at 15·5°. From these data, the weight and volume of the tar are readily calculated, and hence the specific gravity.

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[1] "Methods of Analysis used in the Coal Tar Industry," by a Committee of American Coal Tar Chemists, under the presidency of J. M. Weiss, *J.I.E.C.*, 1918, 10, pp. 732, 817, 911, 1006. Standardised methods are given for the analysis of crude and distilled tars, middle oil, light oil, and benzols. The apparatus and methods are very fully described.

As previously mentioned, the specific gravity of tars which consist mainly of aromatic hydrocarbons is usually above 1·000; if under this figure, the tar is generally not suitable for the production of substances for synthetic purposes. The mean specific gravity of gas tar from horizontal or sloping retorts is, according to Köhler, 1·155; from vertical retorts, according to Bueb, 1·100. Most coke-oven tars have specific gravities of the same order, *i.e.*, lying between 1·1 and 1·3. The specific gravity is influenced to some extent by the amount of free carbon in the tar.

Free Carbon.—Kraemer and Spilker warm 1 part of tar with 3 parts of aniline, and pour the liquid on to an unglazed porous tile, which will absorb the soluble part of the tar and the aniline, leaving the insoluble carbon as a flaky mass. The latter is transferred, without loss, to a weighed watch glass, and weighed after drying in a steam oven for several hours. A more accurate method is recommended by Weiss (footnote, p. 42). The dry tar is passed hot through a 30-mesh sieve to remove foreign substances. 10 grams are weighed in a 100 c.c. beaker and digested with pure toluene at 90° to 100° for not more than 30 minutes, stirring frequently. A filter cup is prepared as follows:—Two 15 cm. circles of Whatman No. 50 filter paper, one of which has been cut down to 14 cm., are folded symmetrically round a stick of about one inch diameter, the smaller paper inside, so as to form a cylindrical cup about two and a half inches long; the cup is soaked in benzene to remove grease, dried in a steam oven and kept in a desiccator. The toluene tar mixture is poured through the filter cup, which has been wetted with toluene, returning any filtrate which may not be clear to the cup, and the beaker is washed out with toluene till clean, using a camel-hair brush to detach solid particles; all washings are passed through the filter cup, after which the latter is washed with pure benzene and allowed to drain. A filter paper or alundum cap is then placed on the cup, and the whole placed in an extraction apparatus (see p. 87) and extracted with pure benzene until the descending benzene is colourless. The cup is then removed, the cap taken off, and the cup dried in a steam oven and weighed in a weighing bottle, the increase in weight being the matter insoluble in benzene. Approximate results may be obtained working on tar which has not been dehydrated, if not more than 5 per cent. of water is present.

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[1] Weiss (footnote, p. 42) describes a distillation method involving the use of special apparatus for the estimation of water in tar and for the dehydrating of tar.

Falciola[1] adopts a process based on Ceruti's method, gradually introducing 5 grams of tar into 125 to 155 c.c. of olive oil at 140° to 160°, heating to 180° to 190°, then cooling to 150° and filtering through a tared filter. The residue is similarly treated with 50 to 70 c.c. of oil, and transferred to the filter by washing with ether; the filter and contents are washed with ether, dried, and weighed.

Falciola finds 9 to 26·7 per cent. of free carbon in Italian tars; the limits usually given are 10 to 30 per cent. The formation of free carbon is encouraged by high distillation temperatures; gas tars are therefore liable to contain more of this constituent than coke-oven tars. The carbon, which is deposited as a hard graphitic mass, remains in the retort, forming part of the coke; part of the finely divided carbon, which is formed at the same time, passes over with the tar, and part remains in the pitch after the volatile portions have been distilled off. It is possible to estimate roughly the amount of pitch which may be expected from a tar from the amount of free carbon which is contained in the latter, as good pitch of medium hardness, as produced by most tar distillers, contains, on an average, about 28 per cent. of free carbon. Tars containing a large proportion of this constituent are apt to froth on distillation.

Distillation Test.—This test is carried out with a view to ascertaining the nature and approximate amounts of the various fractions obtainable on distilling the tar on a large scale; as it is hardly possible to reproduce the conditions obtaining on a manufacturing scale in an ordinary small scale laboratory operation, it is customary to distil from 3 to 5 kilos of tar in specially constructed metal vessels. Such methods will not be described here, as they cannot be conveniently carried out with the ordinary laboratory equipment ; for a detailed description, including also the estimation of water in tar, see Lunge's "Technical Methods of Chemical Analysis."

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[1] *Ann. Chim. Applic.*, 1917, 7, p. 152; abs. *Analyst*, 1917, p. 246.

The following small scale operation, devised by B. Nickels, is described, as it will afford an opportunity of studying the behaviour of coal tar on distillation, and provide further material for analytical work : 250 c.c. of tar are introduced into a glass retort of 750 c.c. capacity ; the retort is placed on a cup-shaped piece of coarse wire gauze which rests in a circular hole in a piece of sheet iron. No thermometer or special condensing apparatus are necessary, and the heat is supplied by means of a powerful Bunsen burner which is protected from draughts by asbestos screens. The retort is covered by a dome, which may be made from a tin can, a trifle larger than the bulb of the retort, by cutting out a piece from its side in order to make room for the neck. The heating is regulated so that the distillate falls in drops in rapid succession ; towards the end, it will be necessary to heat strongly, so that the wire gauze becomes red hot ; when the pitch begins to intumesce, the heating is discontinued. If the distillate solidifies in the tubulure, it is melted down by cautiously heating with a Bunsen flame. The distillate is divided into the following fractions :—

(1) Ammoniacal liquor and total light oils.
(2) Middle and heavy oils.
(3) Anthracene oils.

Fraction (1) is collected in a graduated cylinder, which is changed for a small weighed beaker as soon as a drop of the distillate solidifies when dropped into water. The amount of total light oils will be too small for examination; after reading off their volume, they are separated from the ammoniacal liquor, which may then be titrated by means of standard acid, in order to estimate the ammonia. The second fraction will, at first, consist largely of solid naphthalene, and will afterwards become more liquid. When a drop of the distillate, collected on a cold metal surface, deposits yellow or greenish amorphous matter, the receiver is changed for a second small weighed beaker in which the last fraction is collected until the heating is discontinued. The second and third fractions may be weighed, the former being assayed for phenols, or tar acids, and the latter for anthracene, by methods subsequently described. When the retort is nearly cold it is plunged into cold water; the pitch will then shrink, so that it may be removed in a lump on breaking the retort, and weighed. Knowing the volume of the tar distilled and its specific gravity, the percentage amounts of the various constituents weighed may readily be calculated.

During the first stages of the distillation, the presence of water may cause bumping; should the inconvenience caused thereby be serious, the tar should first be freed from water by the method described under the determination of the specific gravity.

The above process must, of course, not be regarded as an exact analytical operation; apart from the fact that unavoidable losses of the lighter constituents will occur, it must be borne in mind that the relative amounts of the various fractions may vary according to the rate of the distillation.

Weiss (footnote, p. 42) describes a more accurate laboratory method for distilled tars; being accurately standardised in every detail, this method is far better adapted for obtaining comparable results than the foregoing one. Briefly, it consists in distilling 150 c.c. of tar at the rate of 1 c.c. per minute from a 250 c.c. distilling flask of special dimensions, resembling the Engler flask (see Fig. 15, p. 212), through a condensing apparatus of special dimensions; an accurate nitrogen thermometer is placed as shown in Fig. 15. Fractions are collected as follows in graduated cylinders:—up to 110°, 110° to 170°, 170° to 235°, 235° to 270°, 270° to 300°. The flask is heated by a naked Bunsen flame, and shielded from draughts by a cylinder of metal. For further details, the original paper may be consulted.

THE EXAMINATION OF FIRST RUNNINGS AND LIGHT OILS.

When these two fractions are collected together, as is often the case, they are known as "first light oils," "crude or once run naphtha," or "total light oils." For the sake of clearness, the latter term will be employed to signify the total distillate, exclusive of ammoniacal liquor, resulting from the first distillation of coal tar, up to the point at which the distillate becomes heavier than water. The products coming under this heading are usually yellow to dark brown, mobile liquids of a penetrating smell recalling ammonium sulphide, carbolic acid and naphthalene, at the same time. A green fluorescence is sometimes observable, owing to tar carried over in small quantities during the distillation. The total light oils are usually completely volatile below 180° on redistillation, and have specific gravities ranging from 0·910 to 0·950.

According to Kraemer and Spilker (Muspratt-Bunte's "Chemistry," Vol. VIII., p. 16), the composition of the total light oils is as follows :—

Phenols, consisting of phenol, cresols (chiefly meta cresol) and small quantities of xylenols, 5 to 15 per cent.

Bases, chiefly pyridine and its homologues, 1 to 3 per cent.

Sulphur compounds, consisting of carbon disulphide, thiophen and its homologues, 0 1 per cent.

Nitriles, such as aceto and benzo-nitriles, 0·2 to 0·3 per cent.

Neutral oxygen compounds, such as acetone and coumarone, 1·0 to 1·5 per cent.

Hydrocarbons, 3 to 5 per cent. being olefines from hexylene and upwards, 0·5 to 1·0 per cent., paraffins beginning from hexane, and 1·0 to 1·5 per cent., unsaturated compounds which combine with bromine at the ordinary temperature, such as cyclopentiadenes and the hydrobenzenes.

The remaining 80 per cent. consists of aromatic hydrocarbons, of which about four-fifths belong to the benzene series, and one-fifth to the naphthalene and other series, including hydrocarbons of higher molecular weight. The benzene hydrocarbons consist approximately of benzene, 100 parts ; toluene, 30 parts ; xylenes, 15 parts ; trimethyl benzenes, 10 parts ; tetramethyl benzenes, 1 part ; together with traces of higher methyl and ethyl benzenes. Paraffins, olefines, and naphthenes may be present in varying amounts, but should be practically absent from oils which are to be worked up specially for aromatic hydrocarbons.

Although, as will be gathered from the above list, the light oils are exceedingly complex mixtures, the tests to which they are usually submitted in the laboratory are limited to (*a*) the determination of specific gravity, (*b*) fractional distillation, and (*c*) the estimation of phenols. These tests will, at any rate, enable the analyst to distinguish between first runnings, light oils and total light oils; in order to fully understand the significance of the results obtained, it will, however, be necessary to have considerable experience in dealing with the products in question, both in the laboratory and on a manufacturing scale. Further chemical tests are applied to the purer products obtained from the first runnings and light oils on distillation. (See below, under "Benzols, Commercial Benzene, Toluene and Xylene.")

Specific Gravity.—This may be determined by means of an ordinary hydrometer or specific gravity float, or, more accurately, by means of a Westphal or Mohr balance. An instrument of this type is shown in Fig. 6. For use, it is mounted as shown, the thermometer plummet being suspended in boiled distilled water at 15·5° from the end of the graduated beam by means of a fine platinum wire; equilibrium is established by means of the adjusting screw, with weights corresponding to a specific gravity of 1·0000 on the beam. Each of the largest weights corresponds to 0·1 in the figure for the specific gravity; one of these may therefore be suspended from division 10, or two of them from division 5 when making this preliminary adjustment. The vessel containing the water is then removed, and the plummet is wiped dry and suspended in the liquid of which the specific gravity is to be determined, without disturbing the adjustment of the instrument. The temperature of the liquid should be the same as that of the water which it has replaced. The pointer of the balance is then brought back into its original position by adjusting the necessary weights on the graduated arm, taking care that the suspending wire is immersed to the same depth as before; as each weight weighs ten times as much as the next smaller size, the specific gravity may be directly read off without any calculation. If the specific gravity float is used, it will also be necessary to have the liquid at 15·5°.

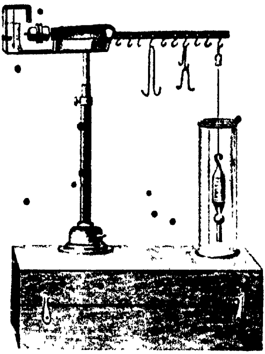
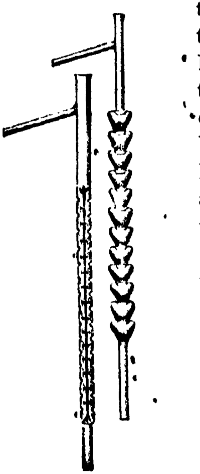


FIG. 6. Westphal Specific Gravity Balance.

The specific gravity of the total light oils usually lies between 0·910 and 0·950. The average specific gravity of English first runnings is 0·900, and that of light oils 0·975.

Distillation Test.—For the carrying out of this test, several methods are in use, and it is, therefore, necessary to exercise some caution in comparing results obtained by different analysts. The most scientifically accurate method consists in distilling the liquid from a flask fitted with an effective fractionating column. When such an appliance is inserted between the distillation flask and the condenser, the higher boiling portions of the liquid which might otherwise be carried over with the vapours, are condensed and run back into the flask; a more complete separation of the constituents according to their boiling points is thus obtained. For the present purpose, Lunge recommends the use of a three bulb Linnemann apparatus or the Hempel tube. Sidney Young's "Pear" and "Rod and Disc" forms, figured here, are also very efficient for the fractionation of benzols.



FIGS 7 and 8

Sidney Young's "Rod and Disc" and "Pear" Fractionating Columns

The thermometer should be placed so that the upper end of the bulb is on a level with the bottom of the side tube of the column; it should be graduated in fifths of a degree and tested by comparison with a standard thermometer within the range required, and corrections, if any, noted; before use, it may be tested in steam from water boiling in a distilling flask, in which case the correction will include that for barometric pressure; if the pressure should have altered, the correction may be made at the rate of 0·47° per 10 mm. A straight Liebig condenser, at least 18 inches long, is used, and the distillate is caught in graduated cylinders which have been calibrated by comparison with an accurate burette. The flask is heated by a small naked flame, and protected from draughts by means of a cylindrical roll of fine wire gauze. Distillation is usually carried out at the rate of one to two drops per second. 300 c.c. of the liquid may be distilled from a 500 c.c. flask, and the volume of the distillate noted every 10°, from 80° and onwards.

From the results thus obtained an approximate idea may be formed as to the composition of the liquid, which will give some indication of the portion of the coal tar distillate of which it is composed. Working with larger quantities, preferably freed from carbon disulphide, as described below, benzene, b.p. 80·2°, and toluene, b.p. 110·7°, may be separated in a state of purity, or fractions may be obtained for analysis as described under benzols. Ortho, meta and para-xylenes, boiling at 142°, 139° and 138° respectively, are obtained as a mixture; they cannot be separated by fractional distillation.

Good first runnings should, according to Lunge, yield at least 10 per cent. by volume, below 100°, and when the product collected up to 130° is redistilled, it should yield at least 25 per cent. of its volume below 100°. First runnings yield, on an average, about 78 per cent., by volume, below 171°.

Light oils should yield little below 120°, and about 30 per cent. by volume, between 120° and 171°. All which comes over above the latter temperature belongs, properly, to the carbolic oil fraction. If an appreciable amount distils below 120°, the oil probably contains first runnings, while if the total yield up to 171° falls considerably below 30 per cent., a portion of the coal tar distillate, properly belonging to the carbolic oil, has probably been allowed to run into the light oil.

Other methods for examining first runnings and light oils, involving distillation, are given under the heading

"*The Examination of Benzols, etc.*" These also include the estimation of paraffins and carbon disulphide.

Determination of Phenols.—The following rapid method does not yield results of great accuracy, but may conveniently be adopted in technical work. It is based on the solubility of the phenols, and the insolubility of the neutral oils in caustic alkali solution. 50 c.c. of the sample are introduced into a graduated glass-stoppered cylinder of about 250 c.c. capacity, and 100 c.c. of a 9 per cent. solution of sodium hydroxide are gradually added. The whole is well mixed by shaking, and allowed to stand until it has separated into two well-defined layers; the volume of the neutral oils is then read off and subtracted from the original volume of the sample; the difference is taken as an approximate measure of the phenols present. A more accurate reading is got by adding a volume of petroleum ether equal to that of the sample and deducting this from the final reading.

More accurate methods for estimating phenols are given below, when dealing with the purer products (p. 55).

Light oil is used as such, for making varnishes for wood and iron, and occasionally also as an illuminant and as a solvent for pitch. By far the most important use of light oil, as well as first runnings, is in the manufacture of the "benzols" of commerce, from which benzene, toluene and xylene may be produced in a state of purity for the manufacture of colours and other valuable products.

Before redistillation, the first runnings or light oil are washed, first with dilute sulphuric acid to remove pyridine bases, then with concentrated sulphuric acid to remove olefines and other unsaturated compounds such as coumarone, indene, cyclopentadiene, etc., which are converted into resinous bodies, and finally with dilute alkali to remove phenols.

	Percentage Distilling below				Sp. Gr. at 15·5° C.	Approximate Composition
	100° C.	120° C.	130° C.	160° C.		
90 per cent. benzol.	90	—	—	—	0·880 to 0·888	70 per cent. benzene, 24 per cent. toluene, traces of xylene, 4—6 per cent. carbon disulphide and light paraffins, etc.
50 per cent benzol.	50	90	—	—	0·880 to 0·872	Chiefly toluene and xylene with a little benzene.
30 per cent. benzol.	30	90	—	-	0·875	Chiefly toluene and xylene.
Solvent naphtha	*Nil*	-	8- 30	90	0·875	Chiefly xylene and higher homologues with a little naphthalene and paraffins, etc.
Burning naphtha.	*Nil*	—	—	30	0·885	Chiefly xylene and higher homologues; naphthalene and paraffins, etc.

The chief products resulting from the distillation of the washed light oils of tar are set out in the above table, together with their behaviour on further fractionation, specific gravities and approximate compositions. The results quoted here have been collected from data given by Lunge and Allen.

THE EXAMINATION OF BENZOLS AND COMMERCIAL BENZENE, TOLUENE AND XYLENE.

The tests commonly applied to these products, some of which are described in the accompanying table, are (*a*) the determination of specific gravity; (*b*) the investigation of behaviour on fractional distillation; (*c*) the estimation of carbon disulphide; and (*d*) the estimation of non-nitratable hydrocarbons or paraffins. Carbon disulphide occurs chiefly in 90 per cent. benzols (see table), and only in comparatively small amounts in the higher boiling benzols; its presence in appreciable quantities, in benzene which is to be nitrated, is objectionable. Light hydrocarbons, including olefines, consisting mainly of pentene, and paraffins, occur chiefly in the low-boiling benzols, though higher open chain hydrocarbons are often met with in the heavy benzols and in commercial toluene and xylene. The presence of such impurities in appreciable amounts is highly objectionable, causing trouble in the nitration process, and lowering the yield of amines. Commercial benzols are sometimes adulterated with petroleum spirit, which consists chiefly of heptane, or with shale naphtha, which contains about 50 per cent. of olefines, mainly heptene, and about 50 per cent. of paraffins.

In addition to the above-mentioned tests, the following tests for determining the relative purity of benzene, toluene and xylene are described: (*e*) the bromine absorption test, and (*f*) the sulphuric acid test. Under the heading Xylene will be described the estimation of meta-xylene in presence of its isomers.

The value of a benzol depends, first, on its relative freedom from the undesirable impurities mentioned above, and second, on the proportion of benzene or toluene present, the latter substances being two of the most valuable constituents of coal tar. As regards the xylenes, only the meta isomer is of any value to the colour manufacturer, the ortho and para isomers being looked on as undesirable impurities.

Specific Gravity.—This is best determined by means of the Westphal balance, as described on p. 50.

The indications afforded by this test are not always of a very definite nature. In the case of 90 per cent. benzol, a high specific gravity may generally be taken as pointing to the presence of an appreciable quantity of carbon disulphide. The specific gravity of coal tar naphtha should never be below 0·870; if lower, light paraffins are probably present, as the specific gravity of petroleum spirit is, at the most, only very little over 0·700. On the other hand, the effect of the presence of carbon disulphide on the specific gravity counteracts that of the light paraffins, so that a sample containing both of these impurities may well have a normal specific gravity. (See also following section.)

Fractional Distillation.—This may be carried out as described under the heading of Light Oils and First Runnings; the volume of the distillate should be noted at the temperatures given in the last table, for comparison of results.

The products dealt with under the present heading distil within narrower limits of temperature than the crude coal tar distillates from which they are derived, the constancy of boiling point of a sample naturally depending on the number of times which it has been redistilled with a view to purification. The terms "90 per cent. benzol," "50 per cent. benzol," etc., refer to the proportion of the original sample which distils below and up to 100°. According to Allen, a good 90 per cent. benzol should not distil below 80°, and only yield 20 to 30 per cent. below 85°, and not more than 90 per cent. below 100°. It should be wholly distillable below 120°. If, say, 35 to 40 per cent. distils below 85°, too much carbon disulphide or light hydrocarbons are probably present.

50 per cent. benzol should distil wholly below 130°, and should yield 50 per cent. below 100° and 40 per cent. between 100° and 120°.

30 per cent. benzol should yield 30 per cent. below 100° and 60 per cent. from 100° to 120°.

Pure benzene, toluene and xylene of commerce distil within 1° C.

A good deal of work has appeared recently on the analysis of benzols by distillation. It will only be possible to refer to a few of the methods which have been described, and reference must be made to the original papers for the tables and graphs by which the results are to be interpreted, and which are based on results obtained with mixtures of known composition. The methods are good examples of standardised processes suitable for the examination of certain technical products.

The following methods require no special apparatus except an Engler distillation flask (see Fig. 15), which, however, is a stock article with most dealers; a twelve-bulb Young's pear fractionating column may also be required. 100 c.c. of the sample are distilled from the Engler flask (without column) at the rate of 7 c.c. per minute; the directions and precautions to be observed as regards condenser, thermometer and graduated cylinders, are as described under the distillation test, page 52.

Spielmann and Wheeler[1] analyse *commercial benzols* as follows:—The volume in c.c. distilling up to 90° is determined as just directed, and from this the percentage of *toluene* is deduced by referring to the toluene curve. Normal amounts of carbon disulphide and paraffins do not interfere with the process, and results are reliable up to 20 per cent. of toluene. Carbon disulphide is estimated by the specific gravity method described on p. 64, and the paraffin content is deduced from the specific gravity by referring to the paraffin curve; as mentioned above, paraffins lower the specific gravity, so that the amount by which the specific gravity is lower than it should be for a mixture of benzene and toluene of the determined composition gives a measure of the paraffins. *Benzene* is determined by difference.

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[1] *J.S.C.I.*, 1916, 35, p. 396.

The foregoing method is only applicable to good average quality benzols containing not more than 20 per cent. of toluene, 3 per cent. of carbon disulphide and 6 per cent. of paraffins. It has been extended by Spielmann and Jones[1] for the analysis of first runnings, which differ from benzol in containing 10 or 15 per cent. of carbon disulphide and up to 15 per cent. of paraffins. The general methods of analysis and interpretation of results are as just described. (See also page 62 for carbon disulphide.)

Colman[2] determines toluene in commercial toluols and in solvent naphtha as follows:—100 c.c. of *toluol* are distilled from an Engler flask (without column) at the rate of 7 c.c. per minute, observing the directions and precautions given under the distillation test, p. 211. The flask and condenser should be washed out with the toluene and allowed to drain just before making the test. The toluene is poured into the flask from a 100 c.c. measuring cylinder which is just allowed to drain. When the distillation temperature has reached 105°, the flame is removed from the flask, and the condenser is allowed to drain. The receiver is then changed, and the distillation continued till a temperature of 117° is reached, when the flame is again removed and the condenser allowed to drain. The residue in the flask is cooled and poured into a 100 c.c. measuring cylinder. The two fractions and the residue should amount to 99 5 c.c. From the amount distilling below 105° and above 117°, the amount of toluene is found by means of the table, a correction being applied for paraffins. The paraffin correction is estimated by distilling 100 c.c. of the sample from a round-bottom flask of 150 to 200 c.c. capacity, fitted with a Young's twelve-pear column, at the rate of one drop per second. The distillate from 107° to 115° is collected, and its specific gravity is taken at 15·5°. For every 0·001 below 0·868 a reduction of three-quarters of one per cent. is made in the percentage of toluene found by distillation.

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[1] *J.S.C.I.*, 1916, 35, p. 911.

[2] *Journ. Gas Lighting*, 1915, 129, pp. 196 and 314, abs, *Analyst*, 1915, pp. 166 and 170.

The tables only apply to samples containing from 50 to 75 per cent. of toluene, and to such as yield at least 5 per cent. of distillate below 105° and not more than 50 per cent. above 117°. Small amounts of carbon disulphide, paraffins and cumenes do not affect the results, reasonable accuracy being obtained with the majority of commercial toluols which have been washed with caustic soda and sulphuric acid. Samples which do not conform to the above boiling point limits may be analysed by this method if previously mixed with a definite volume of pure benzene or xylene or both (see paper).

Toluene is determined in *commercial solvent naphtha* as follows :—100 c.c. of the sample are distilled at the rate of one drop per second from a round-bottom 150 to 200 c.c. flask, using the Young column as described above, the flask and condenser having previously been rinsed out with the sample and allowed to drain. The distillate up to 138° is collected and measured in a 100 c.c. cylinder as described above ; if less than 35 c.c., a further 100 c.c. of the sample is distilled—the distillates being combined ; if still less than 35 c.c., the sample may be taken as practically free from toluene. If the distillate measures above 35 c.c., then 35 c.c. of it are mixed with 50 c.c. of pure toluene and 15 c.c. of pure benzene, and the 100 c.c. of mixture thus obtained is analysed by distillation direct from an Engler flask as described above. The percentage of toluene found from the table is subject to the paraffin correction, made as described above ; deducting the 50 c.c. of toluene added from the total found, the amount of toluene in 35 c.c. of the distillate is arrived at ; a simple calculation gives the percentage of toluene in the total distillate which represents 100 or 200 c.c. of the original sample as the case may be.[1]

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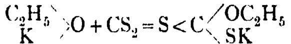
[1] Other methods recently published are as follow :—" Determination of Xylene in Solvent Naphtha," Spielmann and Jones, *J S C.I.*, 1917, 36, 489 ; " Estimation of Toluene, Application of Method to Benzene and Xylene," James, *ibid.*, 1916, 35, p. 236 ; " Estimation of Benzene and Toluene in Commercial Mixtures," Edwards, *ibid.*, 1916, 35, 587 ; " Analysis of Commercial Pure Benzols," Butler Jones, *ibid.*, 1918, 84, 324 T ; " Determination of Benzene, Toluene, etc., in Coal Tar and Similar Products," Colman and Yeoman, *ibid.*, 1919, 38, 57 T ; " A New Method for the Determination of Toluene in Commercial Toluols," Northall Laurie, published by H.M. Stationery Office. See also Weiss, footnote, p. 42.

(*c*) *Carbon Disulphide.*—The process for the estimation of this constituent, now to be described, depends on the formation of potassium xanthate by the interaction of carbon disulphide and potassium ethoxide as follows:—



$$\begin{matrix}C_2H_5\\K\end{matrix}\Big>O + CS_2 = S<C\Big<\begin{matrix}OC_2H_5\\SK\end{matrix}$$

The precipitated xanthate is separated and estimated by titration with standard copper sulphate solution, or analysed for sulphur.

If the sample should be turbid owing to the presence of water, it should first be dehydrated by shaking up with plaster of Paris and filtering. For the estimation, Kraemer and Spilker recommend the following process: 50 grams of benzol are mixed with 50 grams of alcoholic potash, made by dissolving 11 grams of potassium hydroxide in 90 grams of absolute alcohol; the whole is then shaken occasionally during five to six hours. If carbon disulphide is present, the xanthate will separate out in yellow silky needles. The latter are separated in aqueous solution by shaking the mixture with 100 c.c. of water in a separating funnel and washing the remaining benzol with successive small quantities of water which are added to the main aqueous extract.

The xanthate may then be estimated in the aqueous solution as follows: A solution containing 12·475 grams of crystallised copper sulphate per litre is prepared; 1 c.c. of this corresponds to 0·0076 gram of carbon disulphide in the titration described below. The aqueous xanthate solution is acidified with dilute acetic acid, whereupon it must immediately be titrated with the copper solution, as free xanthic acid decomposes spontaneously. A brown precipitate of cuprous xanthate will be formed at first; on the addition of more of the copper solution, this precipitate is transformed into the bright yellow cupric xanthate. The end point is reached when a drop of the solution taken out on a glass rod, and placed beside a drop of dilute potassium ferrocyanide solution on a piece of filter paper, produces a red mark at the point of contact of the liquids; the amount of copper solution which has been added should then be in slight excess of that required to interact with the whole of the xanthic acid present, to form the insoluble cupric xanthate. The amount of carbon disulphide in the sample may then be calculated from the number of c.c. of copper solution used, by employing the factor given above.

The following alternative methods may also be employed:—

(i.) The acidified solution of potassium xanthate is treated with an excess of copper sulphate solution, and the precipitated cupric xanthate filtered off, washed, ignited and determined as cupric oxide or copper, as usual.

(ii.) The potassium xanthate solution is warmed with an excess of potassium hydroxide solution and bromine till perfectly clear; the sulphur, which will now all be present as alkali sulphate, is determined by precipitation with barium chloride in presence of hydrochloric acid in the usual way.

After the removal of the carbon disulphide, the residual benzol should be examined by distillation and tested for its specific gravity; a reduction both in the amount of distillate coming over below 85° and in the specific gravity should be noticeable if the sample contained an appreciable amount of the impurity.

Spielmann and Wheeler (footnote, p. 58) estimate carbon disulphide in commercial benzols by determining the loss in specific gravity which occurs after treatment with alcoholic potash and washing away the xanthate; in the graph, a loss of approximately 0·003 corresponds to 1 per cent. of carbon disulphide, 0·007 to 2 per cent., and 0·010 to 3 per cent. Spielmann and Jones (footnote 1, p. 59) apply this method to first runnings in which the percentage of carbon disulphide is usually much higher than in benzols, by first diluting the sample with five times its volume of benzene free from carbon disulphide.

The quantity of alcoholic potash recommended above is sufficient for the removal of quantities up to 5 per cent. of carbon disulphide. In very exceptional cases only will the percentage of carbon disulphide in 90 per cent. benzol exceed this limit; usually it only amounts to 1 to 2 per cent. In 50 per cent. benzol it may sometimes amount to as much as 1 per cent., while in the higher boiling products, it is either entirely absent or present in traces. The pure benzene of commerce may contain from about 0·1 to 0·3 per cent. of carbon disulphide. As was mentioned above, benzene or toluene which is to be nitrated should be as free as possible from this impurity.

(*d*) *Non-Nitratable Hydrocarbons.*—The so-called "nitrofication test," for the estimation of benzene, and non-nitratable hydrocarbons, by nitrating the benzol, will not be described here, owing to its inaccuracy. The following method, due to Frank, Kraemer and Spilker, is based on the fact that the benzenoid hydrocarbons are converted, at the ordinary temperature, into water-soluble sulphonic acids; the oily residue which is unacted on by the acid may consist of paraffins, naphthenes (*i.e.*, hydrogenated aromatic hydrocarbons) and carbon disulphide.

200 grams of the sample are placed in a separating funnel, and 500 grams of fuming sulphuric acid, containing 20 per cent. of the anhydride, are cautiously added in small amounts, shaking well after each addition. When the whole of the acid has been added, the mixture is shaken for 15 minutes, and then allowed to stand for two hours to separate. The lower acid layer is run off, and the upper layer is treated with two successive portions of 500 grams of the fuming acid as just described. The oily layer is separated off, and the acid extracts are united and run slowly and cautiously on to an equal weight of pounded ice. Care should be taken that the temperature does not rise above 40°. The diluted acid will contain a certain quantity of oily residue, unacted on by the fuming acid, which has either been dissolved by the sulphonic acids, or mechanically removed from the main portion. This is separated by distilling the mixture from a flask, over a free flame, the distillate being caught in a small separating funnel. When, apart from any oil which passes over, 50 c.c. of liquid have been collected, the distillation is discontinued, and the oily portion of the distillate separated from the aqueous layer and added to the main portion. The latter is treated with successive portions of 30 c.c. of fuming sulphuric acid as described above, until no further sensible diminution in volume takes place. The residual oil is washed with a small quantity of distilled water in the funnel, separated carefully, transferred to a tared flask and weighed. If the carbon disulphide has previously been determined, it may be deducted from the

total non-nitratable matter. The remainder will consist of paraffins, and possibly also of naphthenes.

By this method, the presence of an undesirable amount of paraffins, which in some cases may be due to adulteration with petroleum spirit or shale oil, may be detected. Benzols should only contain a few tenths per cent. of paraffins, or at the most, only 1 per cent. Commercial xylenes, however, sometimes contain up to 3 per cent. of this impurity.

The method given for the determination of aromatic hydrocarbons in petroleum (p. 219) may be referred to in connection with the above method and other methods involving the use of fuming sulphuric acid. As this reagent attacks some paraffins, some workers prefer to use acid containing only 3 per cent. of SO_3, while it will be seen that Thole uses 98 per cent. sulphuric acid. It is possible that the method just described might be modified on the lines described on p. 219 with advantage. The paper by Colman and Yeoman (footnote, p. 61) may also be consulted. Here the paraffins are estimated in the fractions up to 90° and from 90° to 140° by the specific gravities of the distillates freed from carbon disulphide, assuming the specific gravities of the paraffins in the two fractions to be 0·73 and 0·74 respectively. It is also necessary to know the proportions of the various aromatic hydrocarbons present, the determination of which is described in the paper.

Weiss (footnote, p. 42) describes a more rapid method which is better suited for technical work than the above. 10 c.c. of the benzol is measured into a Babcock milk bottle (see Fig. 22, p. 271), and 10 c.c. of fuming sulphuric acid containing 20 per cent. of free SO_3 are slowly added, cooling the bottle in ice water and shaking vigorously after each addition. When all the acid has been added, the bottle is removed from the ice bath and shaken until the temperature rises to about 40°, and alternately cooled and shaken for fifteen minutes, keeping the temperature below 40°. 10 c.c. more of the acid are then added to the cooled mixture, which is repeatedly shaken and cooled, still keeping the temperature below 40°. Finally, the mixture is allowed to stand at about 35° for half an hour after which the bottle is again cooled in ice water; water is then added cautiously in small quantities at a time, through a funnel which may be made by drawing out a test tube, so that the water will enter below the surface of the acid. During this part of the process, the bottle is shaken and cooled alternately as above. When sufficient water has been added to bring the level of the liquid well within the graduated portion of the bottle, the bottle is whirled for five minutes in a milk centrifuge (see p. 269). The volume of the paraffins is read off in terms of the larger division on the neck of the bottle, neglecting any solid sulphone which may have collected between the two layers. Twice the number of these divisions gives the percentage of paraffins by volume in the sample.

The following tests are sometimes applied in order to determine the relative purity of commercial benzene and toluene or benzols:—

Bromine Absorption Test.—This test, devised by Frank, Kraemer and Spilker, gives an indication of the amount of hydrocarbons present, which combine with bromine at the ordinary temperature, *i.e.*, unsaturated compounds, which should have been removed during the treatment of the benzol with strong sulphuric acid. (See

p. 54.) It is not applicable to xylene, or to mixtures containing much of this constituent.

A tenth normal bromine solution is prepared by dissolving 9·9167 grams of potassium bromide and 2·7833 grams of potassium bromate in water and making up to 1 litre; 1 c.c. of this solution will liberate 0·008 gram. of bromine on acidification with dilute sulphuric acid.[1]

Five c.c. of the sample are placed in a stoppered bottle of about 50 c.c. capacity, together with 10 c.c. of 20 per cent. sulphuric acid, and as much of the bromide and bromate solution as the sample will decolorise after shaking uninterruptedly for five minutes is quickly added. The end point is indicated when the oil floating on the top shows an orange red after standing for fifteen minutes, and a drop of it momentarily produces a dark blue colour on zinc iodide and starch paper. Preliminary trials must be made in order to determine how much of the bromine solution will be required, for in the actual determination it is necessary that the full amount should be added at once, as directed above.

Pure benzene or toluene of commerce should give a distinct permanent colour after adding only 0·1 c.c. of the bromine solution. Commercial 50 per cent. or 90 per cent. benzols require, on an average, 0·6 c.c. and rarely more than 1 c.c.

Sulphuric Acid Test.—This test is also due to Frank, Kraemer and Spilker; it gives an indication of the relative amount of matter present which will react with strong sulphuric acid at the ordinary temperature. (Compare introductory remarks to the bromine absorption test.)

Five c.c. of the sample are vigorously shaken with

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[1] $5 KBr + KBrO_3 + 6 H_2SO_4 = 6 KHSO_4 + 3 H_2O + 3 Br_2$.

5 c.c. of concentrated sulphuric acid in a stoppered bottle, for five minutes, and then compared with a solution of potassium dichromate in 50 per cent. sulphuric acid, contained in a similar bottle. 50 and 90 per cent. benzols should exhibit a colour like that of a solution containing 0·5 to at most, 1·5 gram of chemically pure potassium dichromate per litre. Xylene will give a colour like a solution containing 1·2 to 2·0 grams per litre, while pure benzene or toluene of commerce should give no colour at all.

According to Colman (footnote 2, p. 59) commercial toluene should comply with the following test :—90 c.c. of the sample shaken with 10 c.c. of 90 per cent. sulphuric acid for five minutes should develop not more than a light brown colour.

Weiss (footnote, p. 42) gives a more elaborate set of standards made from solutions of ferric and cobaltous chlorides, and potassium chromate or dichromate of definite concentrations, or mixtures of these. One ounce French square stoppered bottles of uniform shape and size are used for the standards and the tests ; 21 c.c. of the sample are shaken vigorously for fifteen seconds with 7 c.c. of 96 per cent. sulphuric acid, and the colour is compared with the standards after standing for fifteen minutes. For details of standards see paper.

XYLENE.

The xylene as obtained by the distillation of coal tar contains the three isomeric dimethyl benzenes in varying proportions, together with smaller quantities of the higher benzene homologues and paraffins. Of these constituents, meta xylene is the only one of any use in the manufacture of dyestuffs; the ortho and para isomers are not only useless for this purpose, but have to be removed before the chemical treatment of the meta xylene is proceeded with. Paraffins are a very undesirable impurity and may render the xylene unfit for colour making, if present in large amounts. The following variations in composition of xylenes from English and Scotch tars were found by Levinstein:—Paraffins, 3 to 10 per cent., ortho xylene, 3 to 15 per cent., para xylene, 3 to 10 per cent., and meta xylene, 70 to 87 per cent.

In practice, most of the ortho xylene is usually removed as sulphonic acid in the treatment of the benzol with sulphuric acid (see p. 54); meta xylene may be separated from its isomers by converting the mixed xylenes into sulphonic acids and then steam distilling, when only the meta xylene will be regenerated.

Determination of Meta Xylene and Paraffins in Crude Xylene.—The method described here is due to Crafts. A weighed quantity of xylene, about 10 to 20 grams, is poured on to two and a half times its weight of concentrated sulphuric acid contained in a tube of hard glass; the depth of the xylene layer in millimetres is noted, after which the tube is sealed and heated to 120° for one hour, the contents being well mixed by shaking from time to time. After cooling, the tube is opened, and the contents are treated with three to four times their bulk of a mixture of equal parts of concentrated hydrochloric acid and water, shaken well and allowed to stand for one hour at the ordinary temperature. The insoluble oily layer, which consists of saturated hydrocarbons, chiefly paraffins, is measured in the tube by noting its depth in millimetres, or better still, separated off in a funnel, distilled and weighed. The solvent acid is returned to the tube, which is then resealed and heated to 122° for twenty hours. After this treatment, an oily layer will have been formed which will consist of approximately 97 per cent. of the meta xylene; this may be measured, or separated, distilled and weighed; of the xylene sulphonic acids formed during the heating with sulphuric acid, only that derived from the meta xylene is decomposed by heating with hydrochloric acid under the conditions of the experiment.[1]

Spielmann and Jones (footnote 1, p. 59) determine paraffins in xylene as follows:—10 to 20 c.c. of the sample which has been distilled from 138° to 143° are shaken vigorously for forty minutes with two and a half times their volume of diluted fuming sulphuric acid, one volume of acid containing 22 per cent. of SO_3 to two volumes of 95 per cent. sulphuric acid. The absorption of the aromatic hydrocarbons is carried out in a 100 c.c. flask with a graduated neck; after shaking, the liquid is driven up into the neck by adding 95 per cent. sulphuric acid; the mixture is allowed to stand overnight, and the volume of the paraffins read off.

MOTOR BENZOL.

The National Benzole Association has recently issued the following specification for benzole for use as motor spirit:—*Specific gravity*: 0·870 to 0·885. *Distillation test by flask* (see pp. 51-53): not less than 75 to 80 per cent. at 100°, 90 per cent. at 125°. *Sulphur* (see p. 215):

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[1] For the estimation of ortho and para xylenes and ethyl benzene by a continuation of this process, see Crafts, *Comptes Rendus*, 114, p. 1110.

total not over 0·40 per cent. *Water*: nil. *Colour*: water white. *Rectification test* (cf. p. 69): 90 c.c. shaken with 10 c.c. of 90 per cent. sulphuric acid for five minutes to impart not more than a light brown colour to the acid. *Acids, alkalies and sulphuretted hydrogen*: nil. *Freezing point*: shall not freeze at 25° F. below the freezing point of water.

The necessary tests are described elsewhere. The detection of water, acids, etc., calls for no special description. The analysis of motor spirits containing benzol and paraffin is described in Chapter V.

THE EXAMINATION OF MIDDLE OR CARBOLIC OIL AND ITS PRODUCTS.

The principal constituents of this fraction are phenols and naphthalene, of which phenol itself, *i.e.*, monohydroxy benzene, is the most valuable. Coal tar phenols are often spoken of as "tar acids," commercial cresols as "cresylic acid" and phenol, either pure or crude, as "carbolic acid." At the ordinary temperature a large portion of the naphthalene crystallises out from the oil; at 40°, middle oil is a brownish yellow liquid, smelling of carbolic acid and naphthalene. Its specific gravity at 15·5°, which may be determined as described for crude coal tar, generally lies between 1·00 and 1·03; if under the lower limit an unduly large amount of light oils may be present. The crude naphthalene present amounts to 30 per cent or more. In practice the greater part of the naphthalene is separated by filtration. The remaining carbolic oil, which contains phenol, cresols, xylenols and other higher phenols, neutral tar oils, water, naphthalene, pyridine bases, etc., is either sold as such for disinfecting purposes or varnish making, or it is treated with caustic alkali solution in order to separate the phenols from the neutral and basic constituents. The phenols are recovered from the alkaline solution by precipitation with mineral acid, and worked up for carbolic or cresylic acids of varying degrees of purity, by fractional distillation and other processes; the portion insoluble in alkali is worked up for heavy solvent naphtha, pyridine bases and naphthalene.

Specific Gravity.—The specific gravity of middle oil and crude carbolic acid may be determined as described for tar, or by a hydrometer or Westphal balance; if by either of the two last mentioned methods, it is usually determined at 38°; should it be necessary to work at a higher temperature, a correction of 0·00075 per degree is made.

Free Carbon.—This may be determined in middle oil or crude carbolic acid as described for tar (p. 43).

Distillation Test.—This may be carried out as described under creosote oil (p. 94). Water, if present, may be estimated in the course of this test.

Tar Bases may be determined as described on p. 96 for creosote.

Crude Naphthalene.—500 grams of the middle oil are cooled and the naphthalene is separated by filtration, pressed between filter paper until no longer oily, and weighed. It should distil mainly between 210° and 220°.

CRUDE CARBOLIC ACID

This includes the portion of the middle oil which is liquid at the ordinary or lower temperatures, having been separated from the solid crude naphthalene by filtration. According to Lunge, "it should have an average boiling point of 250°, a specific gravity between 0·99 and 1·01, and contain from 25 to 35 per cent. of phenols, and about 5 per cent. of pyridine bases. It may be examined for (*a*) total phenols and water, and (*b*) phenol, *i.e.*, the monohydroxy benzene.

Total Phenols and Water.—The following approximate method is due to Bach; advantage is taken of the insolubility of the phenols in brine, and their solubility in sodium hydroxide solution.

Fifty c.c. of the sample are distilled from a retort until solid matter begins to come over, the distillate being caught in a clean wide 100 c.c. burette, graduated in fifths of a c.c. and furnished with a glass tap. Before the distillation 25 c.c. of a saturated solution of common salt are introduced into the burette, and the level of the liquid is noted. If the carbolic acid contains no water, the oil separates clearly from the brine, while if the contrary is the case, an emulsion is formed which may, however, be broken down by gently agitating the liquid and allowing to stand. When the layers have become distinct, the level of the brine is read off; its increase in volume is a measure of the water in the sample. The brine is then drawn off, the level of the oil noted and the burette filled to the zero mark with sodium hydroxide solution of specific gravity 1·26; after closing with a cork the contents are mixed by shaking vigorously and then allowed to settle. If the burette was originally clean and free from grease the oil will have separated after half an hour, when its level may be observed. The difference between this and the previously observed volume of the oil gives the amount of phenols in the sample.

The percentage of phenols in crude carbolic acid is very variable, especially as the inferior qualities are often adulterated with neutral tar oils. The percentage of phenols is usually stated in naming the product, *e.g.*, "carbolic acid, 25 per cent.," "carbolic acid, 50 per cent."

There is no satisfactory method of determining the naphthalene or the relative proportions of phenol, cresols and higher phenols in crude carbolic acid, though the following process, due to Chas. Lowe, will furnish results which will give a rough idea of the proportion of phenol present in a sample which has not been adulterated with neutral oils.

Phenol, Water and Light Oils.—This method combines separation by distillation with the determination of solidifying point of the distillate.

100 c.c. of the sample are distilled from a retort, and the distillate is collected in graduated tubes. At first water distils and then an oily fluid; when 10 c.c. of the latter have been collected, the receiver is changed. The volume of the water is read off; if the oily liquid floats on the water, it is light oil of tar; if it sinks it may be regarded as hydrated phenol, containing about 50 per cent. of phenol. The next portion which distils consists of anhydrous phenols; when it measures 62·5 per cent., the receiver is again changed. The residue is wholly cresols and higher phenols. The fraction consisting of 62·5 per cent. is cooled and its solidifying point determined; this should lie between 15·5° and 24°. The proportion of phenol to cresols in the fraction may then be estimated by determining the solidifying points of synthetic mixtures of pure phenol and coal tar cresol. (See below, p. 84.) The solidifying point may be determined as described under "Crystallised Carbolic Acid." (See p. 84.)

The proportion of light oils in crude carbolic acid is usually small, at the most 5 to 6 per cent., hence an appreciable quantity of phenol may be lost in the first 10 per cent. of oily matter distilled. In some cases, therefore, it may be best to take the solidifying point of the distillate coming over between 185 and 195°. When dealing with better qualities, *i.e.*, those containing more phenol, it is recommended to take the solidifying point of the distillate coming over between 180 and 190°, as in such cases the whole of the phenol will naturally come over at lower temperatures. The temperatures referred to are indicated by a thermometer, the bulb of which is placed in the vapour of the boiling liquid.

	Crude Carbolic Acid from Tar from		
	Blackburn.	Manchester.	Manchester.
Water, per cent., by volume	12	13	15
First oil up to 185°, (to be rejected) per cent.	11	11	10
Carbolic acid distilling below 190°, per cent.	48	45	45
Ditto above 190°	13½	17½	17½
Solidifying points of the latter, *i.e.*, 61 to 62 per cent. of the total sample	15°	18°	16¾°

The above table contains results obtained by Watson Smith by the method just described.

The neutral oils may amount to as much as 10 per cent., and the water may lie between 10 and 17 per cent. If an excess of cresylic acid is present, crystallisation is prevented in the determination of the solidifying point: in this case, a second fractional distillation should be made, this time stopping when the thermometer, placed in the vapour, indicates 190°.

The melting and boiling points of phenol and the three cresols are as follows:—

—	Phenol.	Ortho Cresol.	Meta Cresol.	Para Cresol
Melting points ° C. -	42·5—43	30—31	4	36·5
Boiling points ° C. -	182	190·5	200—201	201·8

The relative proportions of the three cresols in coal tar are about 35 per cent. ortho, 40 per cent. meta and 25 per cent. para. The mixture of cresols from coal tar boils from 198° to 203°.

As already mentioned, the above process is only a rough one; the estimation of phenol in the presence of the three cresols is in reality a difficult and complex problem which has recently received the attention of several investigators.[1] The methods are based on specific gravity and solidifying point determinations, the material being distilled through special still heads. They are generally very elaborate. Fox and Barker lay stress on the proper drying and purification of the phenol and cresol mixtures before distillation and examination, pointing out that lack of attention to these points has probably caused the discrepancies among the results of previous investigators. (See p. 81.)

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[1] Fox and Barker, *J.S.C.I.*, 1917, p. 842, 1918, 265 T. and 268 T.; G. H. Sharples, *ibid.*, 1918, p. 109 T.; Knight, Lincoln, Formanek and Follet, *J.I.E.C.*, 1918, p. 9; Weiss and Downs, *ibid.*, 1917, 9, p. 569; Dawson and Mountford, *J.C.S.*, 1918, p. 935.

PURE CARBOLIC ACID AND ITS PREPARATIONS.

The crystallised pure carbolic acids of commerce consist of more or less pure phenol; they may contain small quantities of cresols and higher boiling compounds which produce a red or yellow colour, water and traces of metallic compounds.

Liquefied pure carbolic acid usually consists of about 90 parts of pure phenol and 10 parts of water or alcohol. It may be distinguished from "liquid carbolic acid," which usually consists of cresols and higher homologues by the two following tests (*a*) and (*b*):—

(*a*) *Boiling point.*—Liquefied pure carbolic acid begins to boil below or near 100°, after which the boiling point quickly rises to 185° to 190°, while the product containing cresols will boil at 185° to 209°.

(*b*) *Solubility in water.*—The liquefied product requires at most 18 parts of water to give a clear solution, while cresylic acid is not completely dissolved by even 50 parts of water.

Estimation of Phenol.—The method to be described is Koppeschaar's modification of Landolt's process, based on the formation of the insoluble tribromophenol by the interaction of phenol and bromine in aqueous solution. A known amount of bromine having been added to the phenol solution, potassium iodide solution is added, whereupon an amount of iodine corresponding to the excess of free bromine present is liberated; this is estimated by titration with standard sodium thiosulphate solution, using starch as indicator. The amount of bromine used up in the formation of tribromophenol may then be calculated. It should be noted that this method is only applicable to the products dealt with under the present heading, *i.e.*, pure or nearly pure phenol, or solutions of the latter which do not contain cresols or other substances which also react with bromine water. The following solutions will be required :—

A solution of sodium thiosulphate, corresponding to 5 grams of iodine per litre. (5 grams I = 9·764 grams $Na_2S_2O_3$ 5 H_2O.) This solution may be standardised according to Volhardt's method, as described on p. 128.

Potassium iodide solution.—A 10 per cent. solution of the pure salt in water.

Starch solution.—Freshly prepared by heating half a gram of powdered starch with 50 c.c. of water in boiling water.

Bromine solution.—A solution containing five molecular proportions of potassium bromide to one of potassium bromate, of such a strength that 50 c.c. mixed with 5 c.c. of strong hydrochloric acid and 100 c.c. of water [1] requires for complete decolorisation 86 to 95 c.c. of the thiosulphate solution described above. It may be prepared either by dissolving the pure salts in water, in the requisite proportions, or by adding to a solution of pure sodium hydroxide an excess of bromine and evaporating to dryness; 9 grams of the powdered residue are dissolved in 100 c.c. of water and diluted to the requisite strength after a preliminary titration.

The actual determination of phenol is carried out as follows: 4 grams of the sample, or more if the amount of phenol present is small, are dissolved in or mixed with water, and made up to 1 litre. 25 c.c. of this solution, filtered if necessary, are placed in a bottle provided with a well fitting stopper, of about 400 c.c. capacity. 100 c.c. of the bromide and bromate solution are added, and then 5 c.c. of concentrated hydrochloric acid, in order to set free the bromine. The bottle is immediately closed, shaken and allowed to stand for fifteen minutes. 10 c.c. of the potassium iodide solution are added and the whole is well mixed again. The free iodine liberated by the excess of bromine is estimated by titration with the thiosulphate solution, adding a few c.c. of the starch solution as indicator towards the end of the process.

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[1] See footnote, p. 68. $2\,KI + Br_2 = 2\,KBr + I_2$.
$2\,Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2\,NaI$.

If the standard solutions used are of the strength prescribed above, and the operations carried out as described, then the percentage of phenol in the sample is given by the formula

$$(2a - b) \times 0{\cdot}618,$$

where a = the number of cubic centimetres of thiosulphate solution required by 50 c.c. of the bromide and bromate solution used, and b the number of cubic centimetres required by the iodine equivalent to the final excess of bromine.

Care should be taken that the 25 c.c. of solution used for titration do not contain more than 0·1 gram of phenol.

Lloyd[1] has criticised the above method and proposed an alternative one. Olivier[2] maintains that this criticism is unfounded, and further states that the time allowed for the reaction between the phenol and the bromine may safely be reduced to five minutes provided that the amount of phenol does not exceed 0·09 gram, and that the strength of the bromide and bromate solution is approximately 0·8 tenth normal. The addition of 10 c.c. of chloroform as recommended by Lloyd, enables the end point to be determined with greater accuracy.

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[1] *J. Amer. C. S.*, 1905, 27, p. 16.

[2] *Rec. Trav. Chim. Phys. Pays Bas*, 1909, 28, p. 354, abs. *Analyst*, 1910, 134.

The following directions are given by Fox and Barker (*loc. cit.*) for the estimation of phenol in cresylic acid, the object being to ascertain whether any appreciable amount of phenol, say more than 5 per cent., has been left in the cresylic acid. The directions for determination of solidifying points may be applied to the testing of the purer phenol products (see p. 78), or to the distillates obtained by the preceding process (p. 76).

Determination of Phenol in Cresylic Acid.—If more than traces of neutral oils, naphthalene and bases are present, these must first be removed; 100 c.c. of the sample are shaken with 200 c.c. of caustic soda solution in a separating funnel, and extracted with three successive quantities of 20 to 30 c.c. of ether.

(The neutral oils, etc., may be estimated, if desired, by drying the ether solution with calcium chloride, the latter being washed with dry ether after removal and the washings added to the main portion, evaporating off the ether in a tared flask on the water bath and weighing after heating in the steam oven till all traces of ether have evaporated.)

The caustic solution in the funnel is acidified with sulphuric acid, and the aqueous portion run off from the tar acids during cooling; the aqueous portion is *extracted with ether to remove dissolved tar acids*, and the ether extract mixed with the main bulk of tar acids. The ether solution of tar acids is washed with a nearly saturated solution of sodium sulphate to remove sulphuric acid, and dried with fused calcium chloride which has been recently ignited and cooled in an atmosphere of carbon dioxide. (This precaution is taken as heated calcium chloride may contain a little lime which would combine with the tar acids.) During the following operations, precautions should be taken to prevent the tar acids absorbing moisture. The calcium chloride is washed with dry ether after removal, and the washings are added to the main ether solution. The ether is evaporated off, and the tar acids are distilled from a round flask through a column (Fig. 7 or 8), the condenser being an adapter the small diameter limb of which is a tube 15 inches long, placed vertically. The flask, the globular portion of which is 3 inches high and the neck preferably 4 inches long, is enclosed by an asbestos or tin shield having a slot to admit the supporting clamp, and is heated by a naked flame. Connections are made with new corks as rubber will be dissolved. The thermometer should be accurately calibrated and indicate from 90° to 250° in half degrees; the correction for emergent stem is calculated from the formula $0{\cdot}000143\,(T - t)\,N$ where T = observed boiling point, t = temperature of middle point of portion of stem outside vapour, N = number of degrees of stem outside vapour. The position of the thermometer bulb is as in Fig. 15. The first distillation is carried up to 210° at the rate of 7 c.c. per minute. This fraction is redistilled at the same rate up to 202°, and if this fraction does not amount to more than 5 per cent. (*Case* 1), it may be concluded that the sample does not contain more than 5 per cent. *of phenol, and no further test need be* applied. If the last distillate amounts to more than 5 per cent. (*Case* 2), it is redistilled up to and including 195°, and the resulting distillate is tested for phenol by determination of its specific gravity and the bromine water test.

The *specific gravity* is taken in a pyknometer or by a Westphal balance, if sufficient material is available, at any convenient temperature. The result is corrected to 15·5° by the formula $t - 15{\cdot}5 \times 0{\cdot}0005$.

The *Bromine Water Test* is carried out by placing 0·1 to 0·2 c.c. of tar acid distillate in a glass cylinder of about 100 c.c. capacity, and adding 10 c.c. of water, two or three drops of concentrated hydrochloric acid, shaking, and then adding sufficient of a freshly prepared saturated solution of bromine in water to make the volume up to about 100 c.c. After shaking well the precipitate is allowed to settle; if the sample contained 5 per cent. or more of phenol, a voluminous light precipitate is formed, the cresols by themselves producing a dark oil or heavy granular precipitate which settles quickly. The two types of precipitate are also readily distinguished by microscopic examination.

If the specific gravity at 15·5° is over 1·048, and phenol is indicated by the bromine water test, the following alternative process must be adopted: If the distillate up to 195° amounts to about 50 per cent. by volume of the total (*Case* 3), it is weighed, dried with calcium chloride as described above, and mixed with sufficient pure phenol to give a mixture containing at least 80 per cent. of phenol, which mixture is used for the determination of the freezing point as described below. If the distillate up to 195° is much less than 50 per cent. (*Case* 4), sufficient pure ortho cresol is added to the total material distilled up to 210° to bring the volume of the fraction distilled up to 195° to 50 per cent.; the object of this addition is to cause all the phenol to come over in this first fraction, which is further treated as in Case 3 to get material suitable for the solidifying point determination.

Both in Case 3 and Case 4 a second fraction is collected from 195° to 196·5°; this fraction should show negative results in the two tests for phenol described under Case 3.

Solidifying point.—The reason for adding phenol in Cases 3 and 4 is that mixtures rich in phenol are best suited for solidifying point determinations. About 15 grams of the distilled, weighed and dried tar acids are melted and placed in a 6 by ¾ inch test tube, and this is surrounded by a 6 by 1¼ inch tube and placed in water at 20°. The liquid is stirred regularly, once per second, with a stout copper wire; at the solidifying point the thermometer rises to a maximum and remains stationary for some time. The percentage of phenol is found by reference to the following data, which are average results obtained with mixtures of known composition containing pure phenol and commercial cresylic acid free from phenol: 100 per cent. phenol, 40·1°; 95 per cent., 37·4°; 90 per cent., 34·5°; 85 per cent., 31·7°; 80 per cent., 28·9°. The percentage of phenol in the original sample is readily found by allowing for the added phenol.

CRYSTALLISED CARBOLIC ACID. TESTS FOR PURITY.

Under this heading are described the determination of (*a*) the solidifying point, and (*b*) water.

(*a*) *Solidifying Point.*—This is the most important test for the purity of crystallised phenol. The purest phenol solidifies at 40·9°, while for pure phenol of commerce, it is usual to demand a solidifying point of 39° to 41°. The solidifying point may be determined as described on p. 84. The presence of water or cresols will lower the solidifying point, and if any cresols which may be present are to be determined by this method, the sample should be dried if necessary, as described on p. 82.

(*b*) *Water.*—This constituent may be determined in crystallised carbolic acid by mixing it with five times its weight of dry levigated lead oxide and drying at 70° to 80° until constant in weight. The function of the lead oxide is a mechanical one, the object of the admixture being to facilitate the evaporation of the water.

As little as 1 per cent. of water may be detected by the milkiness produced when the phenol is shaken with an equal volume of chloroform.

Crude carbolic acid, consisting either entirely of cresylic acid, *i.e.*, cresols, or of a mixture of the latter with phenol, is largely used for disinfecting purposes. Very often, cresylic acid is mixed with soft soap and water to form an emulsion, owing to its sparing solubility in water. The analysis of such preparations will be described in the chapter on soap. Pure phenol is used in medicine, and in the manufacture of dyes and picric acid.

THE EXAMINATION OF CREOSOTE OIL AND ITS PRODUCTS.

This portion of the coal tar distillate is a viscous, greenish-yellow fluorescent liquid which partially solidifies on cooling, owing to the separation of naphthalene. The solid matter amounts to some 20 per cent. Creosote oil has an average specific gravity of 1·04. Its principal constituents are naphthalene, which usually amounts to 20 to 30 per cent. or more, and higher phenols, including cresols, xylenols, naphthols, etc., which amount to 10 to 20 per cent. In addition to these may be mentioned aniline, and other basic compounds such as acridine, cryptidine, and quinoline, and hydrocarbons such as methyl naphthalene, diphenyl, anthracene, acenaphthene, hydronaphthalenes, etc.

On redistillation, a little light oil and carbolic acid are separated, and the two main fractions obtained, *i.e.*, first and second naphthalene oils, are allowed to cool, and the naphthalene crystallising out is removed by filtration and further purified. The mother liquors contain varying proportions of naphthalene and other hydrocarbons, about 10 to 30 per cent. of phenols and smaller amounts of basic substances. The liquors obtained from the more volatile first naphthalene oil are used as disinfectants, sometimes by themselves, sometimes in the form of emulsions with soft soap, or mixed with lime, kieselguhr, borax, or other solid material. They may also be redistilled to yield a product containing about 50 per cent. of phenols which is mixed with zinc chloride solution to form an emulsion for pickling timber. The mother liquors which form the second naphthalene oil are also largely used for preserving timber, *i.e.*, harbour piers, railway sleepers, paving blocks, etc.

The preparations containing soap, such as creoline and lysol, are treated of in the chapter on Soap. Under the next heading the analysis of phenolic disinfecting powders, creosoting liquors and the testing of naphthalene for purity are described.

THE EXAMINATION OF PHENOLIC POWDERS FOR NEUTRAL TAR OILS AND PHENOLS.

Different methods must be employed, (i.) for preparations made from powders which do not combine chemically with the phenols, such as borax or kieselguhr, and (ii.) for those made from lime or other bases forming salts with the phenols. As is well known, the phenols are neutralised by the oxides or hydroxides of the alkali metals or metals of the alkaline earths, but not by alkaline carbonates, borates, etc.

(i.) *Preparations containing non-basic Powders.*—This, as well as the following method for powders with basic constituents, is taken from Allen's "Commercial Organic Analysis" (revised edition).

50 grams of the powder are placed in a thimble of filter paper and extracted with ether in a Soxhlet extraction apparatus. The author would, however, recommend the extraction apparatus designed by Bolton and Revis [1] for all work of this kind. It is more efficient than the Soxhlet type, and more readily procurable at the present time. Fig. 10 shows the general arrangement; the lower part of the inner or extraction tube, of which there should be three sizes to select from, is lightly packed with cotton-wool which has previously been extracted with ether, and a disc of filter paper is placed on top of the wool; the material is placed on the paper. A fluted filter paper is placed in the neck of the outer tube so as to catch the liquid dripping from the inner

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[1] "Fatty Foods." Another simple type is described by Griffiths Jones, *Analyst*, 1919, p. 45.

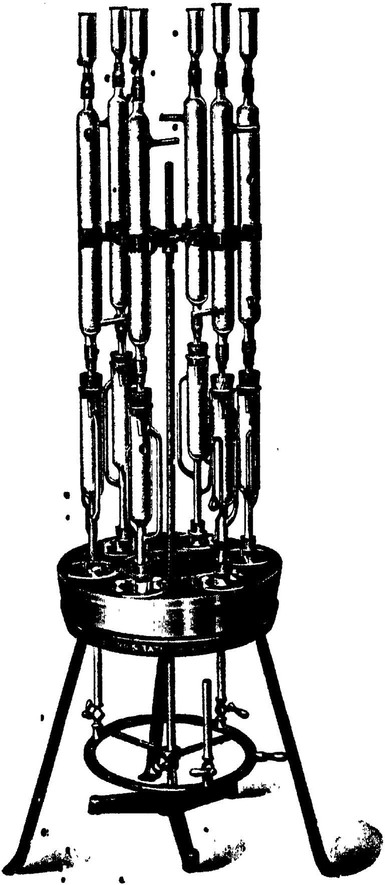

FIG. 9.—Soxhlet Extractors.

tube. The quantity of solvent used should not be sufficient to fill the inner tube. The ether in the flask is boiled as rapidly as is consistent with efficient condensation; electric heating is to be preferred. Bolton and Revis recommend immersing the flask to the shoulder in a water bath in which a 16 c.p. electric lamp is partially submerged. Carbon filament lamps are the best for this purpose, giving more heat than metal filament ones. With the Soxhlet apparatus it is usual to allow four hours for extraction in most cases, but with the extractor described here the process is considerably shorter, partly owing to the fact that the material is heated by the vapour of the solvent passing upwards between the two tubes.

The ethereal solution which contains the phenol is shaken with 20 c.c. of a 20 per cent. solution of sodium hydroxide. (The amount of soda solution may be varied according to the amount of phenols supposed to be present, using 1 c.c. for each 1 per cent.) The ethereal solution is separated off, and the alkaline solution shaken out with two successive portions of ether, in order to extract the whole of the neutral tar oils. The united ether solutions are then shaken out with a small quantity of dilute sodium hydroxide solution in order to remove any traces of phenols which may remain with

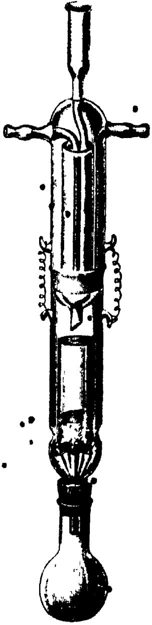

FIG. 10.
Bolton and Revis' Extractor.

the neutral oils, evaporated in a tared flask on the water bath, and weighed, after drying at 100° C. for one hour.

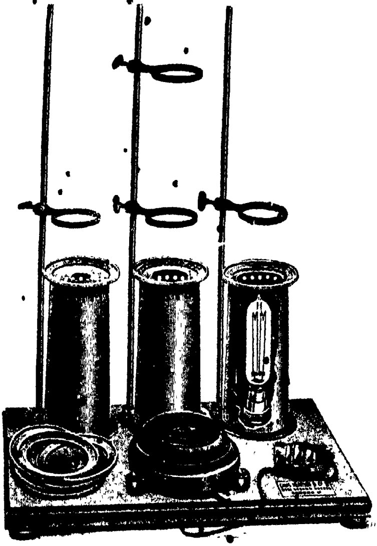

FIG. 11.—Electric Heater.

The united alkaline solutions are boiled down in a flask to about 10 c.c. in volume,[1] transferred to a graduated cylinder or burette, and acidified with dilute sulphuric acid containing one part of the concentrated acid to three parts of water. When the mixture is quite cold the volume of the separated phenols is read off, the weight being estimated on the assumption that 1 c.c. weighs 1.050 grams. If the presence of fatty or resin acids is suspected, the contents of the burette are transferred to a flask and submitted to steam distillation; the phenols will pass over the distillate, while the acids will remain in the flask. The latter may be characterised by their solubility in sodium carbonate solution.

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[1] Phenols being steam volatile and having only weakly acidic properties, may be lost owing to the hydrolysis of their salts unless an excess of caustic soda is present (mass action).

(ii.) *Preparations containing Basic Powders.*—50 grams of the alkaline powder are mixed in a mortar with 5 c.c. of water, after which strong sulphuric acid is added, drop by drop, at intervals and mixed well into the powder or paste by means of a pestle; the addition of the acid should extend over several hours, in order to avoid a sensible rise in temperature; it is continued until the whole mass is distinctly acid. If the resulting product is a paste, it is mixed with sufficient sand to bring it into a granular form; it is then extracted with ether in the Soxhlet apparatus, and the extract is submitted to the same treatment as described above for non-alkaline powders.

According to Allen, good powders should contain from 12 to 18 per cent. of phenols. It is doubtful whether powders containing lime are as efficient for disinfecting purposes as those in which the phenols are present in the free state.

THE TESTING OF CREOSOTING LIQUOR.

Most of the creosote oil distilled is used for preserving timber which is to be used for railway sleepers, telegraph poles, harbour piers, paving blocks, etc. The basic constituents of the oil are believed to be of as much value as the phenols as antiseptics, and, moreover, they are said to form resin-like combinations with the phenols and unsaturated hydrocarbons, which fill up the pores of the wood and protect it from destruction by micro-organisms. Extensive adulteration with lignite, shale and rosin oils, which contain no bases, is therefore objectionable. The naphthalene and other neutral constituents are also believed to be beneficial, for, being introduced into the wood in a liquid state at about 50°, they solidify in the pores and form a protective covering throughout. The amount of pitchy matter should not be high, otherwise the creosote will not penetrate well into the wood, and the pitch should be soft.

The following results of bacteriological tests with organisms which grow on moist wood, by Weiss,[1] are of interest:—Neutral oils of creosote, especially those boiling from 235° to 270°, are strong antiseptics, those of higher boiling point being much weaker, those of lower boiling point being slightly weaker. The coal tar bases and acids of high boiling point are strong antiseptics, the strength of the acids increasing with the boiling point. The solid hydrocarbons, naphthalene and anthracene have low antiseptic value, and paraffins have none at all. The addition of filtered tar does not materially reduce the antiseptic value. Coal tar creosote is a much better preservative than producer gas tar distillates or petroleum residues.

These observations are in accord with Sage's statements[2] that creosote should be "the product of destructive distillation of bituminous coal and free from admixture of other oils"; and that the germicidal value of London creosote is about twice that of crystallised carbolic acid when tested against *Bacillus typhosus.* Again, the American Committee on Wood Preservatives[1] laid down that creosote should consist of all distillate oils coming over between 200° and 300° from tars consisting principally of substances of the aromatic series, and containing well-defined amounts of phenoloids.

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[1] *J.S.C.I.*, 1911, 30, p. 1348. [2] *J.S.C.I.*, 1911, 30, p. 588.

In testing creosoting liquor it is often necessary to adhere to a method described in a contract in which are stated the conditions which the article must fulfil. With such a complex mixture as creosoting liquor, the analytical results often vary considerably with slight variations in the method of analysis, and must therefore be judged with due regard to the process by which they have been obtained.

Specific Gravity.—If taken at 15·5°, this may be determined as described for tar (p. 42). Sage (*loc. cit.*) recommends weighing 100 c.c. at 60° and comparing the weight with the same volume of water at this temperature, or using the Westphal balance with the sample at 60°. Sometimes the determination is made at 38°, but some creosotes may not be entirely liquid at this temperature.

Forrest[2] gives the specific gravities for various creosotes as ranging from 1·03 to 1·16 at 15°, and the specific gravities of other materials for wood paving blocks from 1·10 to 1·17 at 38°. Alleman[3] recommends that the specific gravity should be 1·10.

Fluidity.—Sage recommends keeping the sample at 40° and noting whether it is fluid at this temperature. Another portion is kept at 15° for six hours, stirring and adding a crystal of naphthalene occasionally; the results are returned as fluid, quite solid or some salts (*i.e.*, naphthalene, etc.) deposited.

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[1] *J.S.C.I.*, 1914, 33, 832.
[2] *J.S.C.I.*, 1911, 30, p. 193.
[3] Abs., *J.S.C.I.*, 1914, 33, p. 832.

In view of what was said regarding naphthalene in the introductory remarks to this section, it seems the creosote might well be required at least to conform to the requirement in Tidy's time-honoured specification that it should be completely liquid at 38°, no deposit taking place until it is cooled to 35°. In a test of this sort the conditions for crystallisation should be promoted as recommended by Sage.

Free Carbon.—This may be determined as described under tar (p. 43). The best creosotes contain no free carbon. Sage recommends that the percentage should be not over 0·25 per cent. Any notable amount would indicate adulteration with crude tar, and would very probably interfere with the penetrating properties of the creosote.

Distillation Test (including Estimation of Water).—Sage distils 100 c.c. of the sample from a hard glass retort, measuring 8 oz. up to the bend, and covered in with asbestos or a cut out tin can. The thermometer is placed with the bulb in the liquid, not less than half an inch from the bottom of the retort.[1] The distillates are collected in separate measuring cylinders (see p. 52) as follows:—up to 210°, 210° to 235°, 235° to 270° and 270° to 315°.

The water, if any, coming over with the first portions may be measured; if necessary, solvent naphtha may be added in order to get a clear separation. If more than 1 per cent. of water is present, the sample should first be distilled to remove water, and any oil distilled at the same time separated and returned to the dry oil in the retort.

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[1] Some workers do not agree with this position of the bulb, and prefer to have it in the vapour.

After measuring at 60°, all the fractions up to 315° may be combined for the determination of the tar acids. The pitchy residue is weighed and its consistency noted (see introductory remarks to this section).

Alleman, reporting on the "Qualities and Characteristics of Creosote in Well-Preserved Timbers,"[1] states that such creosotes yielded on an average 32·9 per cent. of distillate below 270° and 66·8 per cent. of oils of higher boiling point. He states that the defects of most modern creosotes are a deficiency of basic oils of high boiling point (see p. 92) and the substitution for them of tar or other viscous substances. (See under Free Carbon. A large pitch residue would also indicate adulteration with tar.) Tar has a low penetrating power. Further, the creosote should contain nothing boiling below 210°, and material for general purposes should yield not more than 50 per cent. below 315°, while material for wood paving blocks should yield not more than 35 per cent. below this temperature.

Tar Acids (*Phenols*).—These are estimated in the combined fractions boiling up to 315°, shaking out with three successive portions of 20 c.c. of 15 per cent. caustic soda solution, acidifying the alkaline extracts with dilute sulphuric acid (1 to 3) in a measuring cylinder, and measuring the liberated phenols at 60°. The oil is shaken with the soda solution in a flask heated on the water bath, after which the mixture is transferred to a separating funnel. Care should be taken to remove as much as possible of the oily matter by means of the separating funnel, after which the alkaline liquid should be boiled vigorously (see footnote, p. 90) to expel the traces of non-phenolic oil which it may retain.

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[1] Abs. *J.S.C.I.*, 1914, 33, p. 832.

Forrest (footnote 2, p. 93) found from nil to 13·6 per cent. of tar acids in various materials for wood preserving. Good creosote should contain at least eight or nine per cent. of these constituents. (See introductory remarks to this section.)

Estimation of the Basic Constituents.—The following method is due to Sadtler: 100 c.c. of the creosote oil are distilled from a retort to the point of coking,[1] and the distillate is agitated with two successive portions of 20 c.c. of dilute sulphuric acid (1 to 3). The acid solution which contains the bases in the form of sulphates is separated off and rendered alkaline by the addition of sodium hydroxide solution. The bases are liberated, partly in the form of an oily layer, and partly in aqueous solution. The oily layer is separated off, and the alkaline liquid is distilled nearly to dryness. The distillate is mixed with the oily extract and the whole is acidified with hydrochloric acid, and evaporated to dryness on the water bath. The residue, which consists of the hydrochlorides of the tar bases, is dissolved in a small quantity of water, and solid sodium hydroxide is dissolved in the liquid till a saturated solution is obtained. The bases may then be separated, weighed, and, if desired, further examined by conversion into platini-chlorides. They may, however, be more conveniently estimated by the following titration process,[2] which was originally devised for the estimation of tar bases in sheep dips. (See Chapter IV.)

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[1] Sage (*loc. cit.*) estimates the bases in the distillate up to 315°.

[2] Bureau of Animal Industry, Bulletin 107., U.S. Dept. of Agriculture.

The acid solution of the bases is filtered if necessary, made up to 300 c.c. with water, and divided into two equal portions in two similar titration flasks of about 300 c.c. capacity. To the contents of one of these flasks is added a drop or two of methyl orange solution, and then semi-normal sodium hydroxide solution until the red tint just disappears, as nearly as can be judged by comparison with the other portion which has been treated with an equal amount of methyl orange. 0·1 to 0·2 c.c. of semi-normal soda solution are then added. This first titration is not quantitative, but is carried out to obtain a standard by which the second portion may be titrated to neutrality.

A neutral solution of the chlorides or sulphates of the bases is thus obtained; any further addition of alkali would produce an alkaline reaction with the methyl orange. Phenol phthalein is now added, and titration is continued to the end point of this indicator; the reason why an alkaline reaction is not observed immediately is that the alkali displaces the tar bases from combination with the mineral acids, and the liberated bases have no action on the phenol phthalein; only when the whole of the bases has been liberated will the alkali added produce a coloration with the phenol phthalein. The number of cubic centimetres of soda solution used between the two indicators, multiplied by 0·079, gives the total amount of the bases in terms of pyridine, or, multiplied by 0·129, in terms of quinoline.

Paraffins (Centrifugal Method).—Weiss (footnote, p. 42) gives the following method for the testing of heavy and middle oils, which is an adaptation of that due to Bateman.[1] The principle is similar to that of the methods described on pp. 66 and 67 for benzols. Ten grams of the sample are weighed into a Babcock milk bottle (see Fig. 22, p. 271), 40 c.c. of 37 N. sulphuric acid (total SO_3, 80·07 per cent.) are added, 10 c.c. at a time, the bottle being shaken for two minutes after each addition. After all the acid has been added, the bottle is kept at 98° to 100° for one hour, shaking vigorously every ten minutes. The bottle is then cooled, filled to the top with ordinary sulphuric acid, and whirled for five minutes in a centrifuge. The unsulphonated residue, consisting chiefly of paraffins, should be a clear transparent oil; it is read off on the scale, the reading in terms of the larger divisions giving the percentage when multiplied by two.

Dimethyl Sulphate Method.—This is an application of Valenta's test (see p. 242). R. M. Chapin[2] recommends the following test in place of or as supplementary to the sulphonation test: 5 c.c. of the sample are shaken in a narrow tube with 8 c.c. of dimethyl sulphate and allowed to stand; the aromatic compounds dissolve in the dimethyl sulphate, and form the lower layer, while the paraffins, if any, will be seen as an upper layer. If a graduated tube is used, the approximate amount of paraffins may be estimated.

This method is not so applicable to mixtures of low boiling point, as the lighter paraffins are somewhat soluble in dimethyl sulphate. The method is, however, useful for the detection of creosotes of doubtful origin, or heavy petroleum oils in admixture with creosote.

Sage (*loc. cit.*) states that English creosote contains very little paraffin, one-half per cent. at the most ; while blast furnace creosote contains 5 to 6 per cent. of paraffins. From what has been said in the introductory remarks to this section, it is obvious that a good creosote should show only a very small sulphonation residue; and practically nothing insoluble in dimethyl sulphate. Appreciable amounts of paraffins will indicate that the creosote was obtained from tar which has been distilled at a low temperature (see p. 38) or from blast furnace tar, or that it has been adulterated with petroleum oils.

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[1] U.S. Dept. Agric. Forest Service, Circular 191, abs. *J.S.C.I.*, 1912, 31, 425.

[2] U.S. Dept. Agric. Circular 167, abs. *Analyst*, 1911, 585.

NAPHTHALENE.

This substance occurs in coal tar to the extent of 5 to 10 per cent., being one of its chief constituents. In the dyeing industry it is of great importance as the source of the naphthols, naphthylamine, phthalic acid, etc. As mentioned above, naphthalene is obtained from the carbolic oil and creosote oil fractions of coal tar ; it is freed from phenols by washing with caustic alkali solution, and sometimes with sulphuric acid, which removes the phenols as sulphonic acids. Further purification is effected by filter-pressing the warm material, and fractional distillation.

The Testing of Naphthalene for Purity.—Naphthalene which is to be chemically treated is required to be as pure as possible. The purest naphthalene melts at 79·5° to 79·8° and boils at 217° to 218° at 760 mm. pressure. The following tests for purity may be applied to commercial naphthalene :

(1) Dissolve in hot pure concentrated sulphuric acid ; the solution should turn only faintly purple or pink. With less pure brands it will turn red.

(2) Pour pure fuming nitric acid on to the bottom of

a desiccator, and place the naphthalene in a watch glass above it, covering up the whole as usual; if the sample remains white for half an hour it is good, and if for two hours, it is excellent. Inferior qualities soon turn pink. After some hours, all samples go yellow, probably owing to the formation of nitronaphthalene.

(3) Phenols may be tested for by boiling the sample with dilute sodium hydroxide solution, cooling, filtering and adding to the filtrate a little bromine water and dilute hydrochloric acid; any phenols which may be present will be precipitated as bromine derivatives.

(4) Quinoline bases may be tested for by dissolving the sample in concentrated sulphuric acid, pouring the solution into water and filtering; on making the filtrate alkaline and distilling, the bases will pass over with the steam and be recognisable by their characteristic smell.

For naphthalene which is to be used as insecticide or for carburetting gas, the tests for purity need not be so stringent.

ANTHRACENE OIL PRODUCTS.

Anthracene oil, which is distilled from about 270° to the point of pitching, is a greenish yellow fluid, turning brown on exposure to air, and boiling from 280° to 400°. On cooling to the ordinary temperature it becomes semi-solid, yielding a crystalline deposit of crude anthracene, amounting to about 30 per cent. of the total. Crude anthracene is an exceedingly complex mixture; in addition to many substances of unknown constitution, it contains the following: naphthalene and homologues, anthracene, methyl anthracene, etc., phenanthrene, acenaphtene, diphenyl, pyrene, fluorene and other hydrocarbons of high molecular weight, phenols of complex constitution, and nitrogen compounds such as carbazole, acridine and imidophenyl naphthyl. The fluid portions of the anthracene oil are of little value; they are either redistilled or used as lubricants. The solid constituents are worked up for anthracene, which is one of the most valuable constituents of coal tar, being the basis of the alizarin dyes. It is present in coal tar to the extent of about 0·3 to 0·9 per cent., and constitutes from 2½ to 3½ per cent. of the anthracene oil. The other constituents of anthracene oil, with the exception of carbazole, are of little or no commercial importance.

After pressing, first cold and then hot, a crude anthracene cake containing some 30 to 40 per cent. of anthracene is obtained. A second crop, crystallised from the anthracene oil at about 15°, yields an anthracene cake containing 10 per cent. of anthracene. The subsequent processes by which pure anthracene is obtained consist mainly in washing the finely divided product with solvents such as naphtha, creosote oil, acetone or liquid sulphur dioxide.

THE EXAMINATION OF CRUDE OR PURIFIED ANTHRACENE.

The value of the product depends, firstly, on the amount of real anthracene it contains, and secondly, on its freedom from undesirable impurities, the chief of which are the higher paraffins and methyl anthracene, which are often present in appreciable quantities in some tars, notably those from Scotland and the north of England. These impurities are very difficult to remove, and if present in appreciable quantities, render the anthracene unfit for alizarin making; the paraffins especially impede the oxidation to anthraquinone, and render the purification of the latter difficult. Carbazole is another objectionable impurity which should be removed as completely as possible. The estimation of anthracene and the detection and estimation of the impurities just mentioned are described below.

Crude unwashed anthracene cake formerly contained about 30 to 40 per cent. of anthracene, but in recent years it has been found possible to obtain a product containing already at this stage from 40 to 50 per cent., or even more, of anthracene.

The Estimation of Anthracene in Crude or Purified Anthracene Cake.—The method usually adopted is known as the "Höchst test," in which the anthracene is quantitatively oxidised to anthraquinone, by means of chromic acid in acetic acid solution, and estimated as such. During the process practically all the accompanying substances are either completely oxidised or converted into products which are easily removed by washing with water or dilute alkaline solution. In order that reliable results may be obtained, the following directions should be closely adhered to:

1 gram of the carefully sampled anthracene cake is treated with 45 c.c. of pure glacial acetic acid in a 500 c.c. flask, fitted with a reflux condenser, and the mixture is kept boiling while a solution of 15 grams of pure chromic acid in 10 c.c. of glacial acetic acid and 10 c.c. of water is added drop by drop; the addition of this oxidising mixture is to extend over two hours, after which the boiling is continued for two hours more. The mixture is then allowed to stand for twelve hours, when 400 c.c. of cold water are added. After another three hours, the precipitated anthraquinone is collected on a filter and washed, first with distilled water, then with

200 c.c. of a boiling 1 per cent. solution of potassium hydroxide, and finally with hot distilled water. The quinone is then transferred, with the aid of a wash bottle, to a dish, dried at 100°, and treated with 10 c.c. of fuming sulphuric acid at 100° for ten minutes on the water bath. The solution thus obtained is poured into a flat dish and kept for twelve hours in a moist atmosphere, say, on a thick layer of moist blotting paper under a bell jar, to absorb water. 200 c.c. of cold water are then added to the contents of the dish; the precipitated anthraquinone is collected on a filter, and washed, first with distilled water, then with boiling 1 per cent. potassium hydroxide solution, and finally with hot distilled water. The residue on the filter is transferred to a platinum dish, dried and weighed; after volatilising the quinone at a gentle heat, the dish is re-weighed with the particles of ash and coal which remain. The difference between the two weights is equal to the weight of the anthraquinone, which, multiplied by 0·856, is equal to the weight of real anthracene in the sample.

The anthraquinone got by the above process should be crystalline, and of a pale yellow colour. An orange or red colour indicates the presence of the quinones of other hydrocarbons, especially those of phenanthrene or chrysene, the latter being recognisable by the indigo coloration which it produces on the addition of concentrated sulphuric acid. The quinone of imido phenyl naphthyl prevents the crystallisation of the anthraquinone; according to Allen, it may be destroyed by longer heating with fuming sulphuric acid. In the presence of methyl anthracene the anthraquinone obtained does not show the usual characteristic needles, but it is more or less felted.

Lewis[1] employs the following method of purifying the anthraquinone in preference to the method of treatment with fuming acid described above. It is more expeditious, and is claimed to estimate accurately anthraquinone in samples contaminated either with large amounts of phenanthraquinone or anthracene. One part of anthraquinone, wet with alcohol, is mixed with two parts of zinc dust and about fifty parts of hot sodium hydroxide solution; the mixture is heated just below the boiling point for five minutes, and then rapidly filtered by suction and washed once with water. The filter paper with the residue is heated with another portion of soda solution, and rapidly filtered into the same flask. The heating and filtration are repeated a third time with a fresh portion of soda solution to ensure that all the anthraquinone has been converted into oxanthranol,[2] which forms a red solution in a solution of caustic alkali of the strength used; if no red colour is formed on boiling the residue for the third time with soda solution, the reduction is known to be complete. The filtrations must be carried out as rapidly as possible for oxanthranol is reconverted into anthraquinone by atmospheric oxidation, a process, which is now applied to the filtrate. The suction flask is cooled in water and shaken while a stream of air is being drawn through it until the red colour has disappeared. The resulting anthraquinone is filtered on a weighed Gooch crucible, washed with water, dried at 110° and weighed.

If the reduction process is carried out too far, some anthranol[3] may be formed; this should be avoided as

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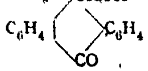
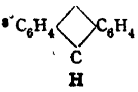
[1] *J.I.E.C.*, 1918, 10, 425.

[2] 

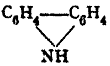
[3] 

anthranol is not converted into anthraquinone by atmospheric oxidation; its presence is detected by any yellow colour in the filtrate from the reoxidation. A green colour in the reoxidised product indicates a reduced compound of phenanthraquinone of unknown structure. The presence of phenanthraquinone leads to high results, but when present in amounts of less than 10 per cent., the error is negligible for practical purposes.

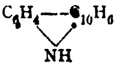
Carbazole,[1] like imido phenyl naphthyl,[2] produces a quinone which interferes with the purification of the anthraquinone; according to Behrens, it is detected by extracting the anthracene with cold ethyl acetate, evaporating the solvent on a watch glass and warming with a few drops of nitrobenzene and phenanthraquinone; characteristic narrow plates of a coppery lustre are got if carbazole is present.

Estimation of Carbazole.—This substance, which occurs in coal tar to about the same extent as anthracene, has lately become of some importance as raw material in the cyanide industry, as well as in the manufacture of dyes. Owing to the weakly acidic nature of the imido group which it contains, it may be separated from the accompanying constituents by heating with potash, when a potassium derivative is formed.

For its estimation, Kraemer and Spilker recommend the following process: the crude anthracene, in a finely divided state, is extracted with warm dilute sulphuric acid, which removes all other nitrogen compounds which are of a basic nature. The nitrogen is then estimated

[1] 

$$\underbrace{C_6H_4—C_6H_4}_{NH}$$

[2] 

$$\underbrace{C_6H_4—C_{10}H_6}_{NH}$$

in the residue by Kjeldahl's process[1] (see p. 21) and calculated to carbazole.

The Determination of Paraffins in Crude Anthracene.—Kraemer and Spilker recommend the following process, which, in theory, is the same as their process for estimating paraffins in benzol (see p. 64): 10 grams of the finely powdered anthracene are shaken with 70 c.c of ether in a 100 c.c. measuring flask for ten minutes, the flask is filled to the mark with ether, and the contents are allowed to settle. 50 c.c. of the clear solution, representing 5 grams of the sample, are introduced into a porcelain dish, the ether is allowed to evaporate, and the residue is dried at 100° for half an hour. After cooling, it is triturated with 8 c.c. of fuming sulphuric acid, containing 20 per cent. of SO_3. The dish is covered with a watch glass and heated to 100° for three hours, with frequent stirring. The contents are then washed into a beaker with 500 c.c. of hot water and, after cooling, passed through a dry filter. The beaker is rinsed out on to the filter, which is washed with water until barium chloride solution no longer produces a precipitate in the filtrate; the filter is then allowed to drain, thoroughly moistened with absolute alcohol, and the paraffin is washed by means of ether into a weighed dish until a few drops of the running ether leave no residue on evaporation. The last traces of paraffin are removed from the beaker by means of ether, which is also passed through the filter and added to the main portion. The total ether solution is evaporated in the dish, the residue dried at 105° for two hours, and weighed as paraffin.

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[1] As these substances contain cyclic nitrogen, it is best to employ the Gunning-Arnold method (see footnote, p. 27).

Good anthracenes should contain little or no paraffin; inferior qualities may, however, contain about 4 to 6 per cent. of this constituent.

REFERENCES.

"Coal Tar and Ammonia," by Lunge 1916. Gurney and Jackson, London.

"Technical Methods of Chemical Analysis," Vol II, Part II., by Lunge, translated by Keane. 1911. Gurney and Jackson, London.

Allen's "Commercial Organic Analysis," revised edition. 1910. J. and A. Churchill, London.

"Chemisch-Technische Analyse," Vol. II, by Post. Fr. Vieweg und Sohn, Brunswick.

"Modern Coking Practice," by J E. Christopher. Vol. II. 1917. Crosby Lockwood, London.

"Coal Distillation," by J E. Christopher. 1915.

"By-Product Coking," by G. S. Cooper. 1917.

"Coal Tar Distillation and Working Up of Tar Products," by A. R. Warnes. 1917 D. van Nostrand, New York. "Gas World," London.

"The Manufacture of Intermediate Products for Dyes," by J C. Cain. 1918. Macmillan, London

"The Chemistry of Coal Tar," by J. W. Fay 1918. D. van Nostrand, New York

CHAPTER III

THE FATTY OILS AND FATS

INTRODUCTORY

THE fatty oils and fats constitute a well-defined group of natural products of outstanding economic importance. Consisting essentially of mixtures of the mixed or simple neutral glycerol esters of the aliphatic acids and other acids belonging to allied series, they may be hydrolysed by the action of caustic alkali with the formation of free glycerol and the potassium or sodium salts of the fatty acids; when the aqueous solutions of these salts, which are generally known as soaps, are acidified with mineral acid, the bulk of the fatty acids are precipitated. These chemical characteristics mark off the fatty oils from other substances also known as oils, *e.g.* the essential oils (terpenes), the petroleum or coal tar oils (hydrocarbons), but indicate a certain relationship with the waxes, which, however, are essentially esters of monohydric alcohols of high molecular weight.

The acids which are most commonly met with in the saponification products of the oils and fats are as follows:—

(1) The aliphatic acids containing an even number of

carbon atoms from butyric acid, $C_4H_8O_2$, to arachidic acid, $C_{20}H_{40}O_2$.

(2) Unsaturated open chain acids yielding dibromo additive compounds, of which the more well-known are oleic acid, $C_{18}H_{34}O_2$ ($CH_3 (CH_2)_7 CH=CH (CH_2)_7 COOH$); rapic acid, $C_{18}H_{34}O_2$; and erucic acid, $C_{22}H_{42}O_2$

$$(C_8H_7 - CH = CH (CH_2)_{11}COOH).$$

(3) Unsaturated acids yielding tetrabromo additive compounds, of which one of the most well-known is linolic acid, $C_{18}H_{32}O_2$.

(4) Acids yielding hexabromo additive compounds, of which the most well-known is linolenic acid, $C_{18}H_{30}O_2$.

(5) Acids yielding octobromo additive compounds, *e.g.*, clupadonic acid, $C_{18}H_{28}O_2$.

In addition to the above, higher or lower aliphatic acids than those mentioned, acids containing uneven numbers of carbon atoms in the molecule, cyclic unsaturated acids and hydroxy acids may be met with in small quantities or in isolated cases. The chemical constitution of many of the acids obtained from the oils and fats is, as yet, doubtful.

By far the greater number of the fatty oils and fats are complex mixtures from which it is generally extremely difficult to isolate chemical individuals; many of the methods for their examination and identification are therefore methods for determining the mean of certain chemical or physical characteristics of their constituents or of the mixtures of fatty acids which have been obtained from them by certain standard methods. Under this heading come the so-called "quantitative reactions" such as the determination of the iodine absorbing power and the saponification value, which are described below.

Chemical methods of a more definite nature may, however, be employed in certain cases; as an example may be mentioned the examination of the small amounts of unsaponifiable matter which are present in all fatty oils and fats. The vegetable oils and fats, or at least all the more commonly known members of this group, contain small amounts of an easily recognised substance known as phytosterol, which is absent from all animal fats: the latter, on the other hand, contain small amounts of cholesterol, which is absent from the vegetable products. Another example is seen in the isolation of arachidic acid from arachis oil. The colour tests, such as those employed for the recognition of sesame and cotton-seed oils, depend on the detection of small amounts of characteristic constituents of these oils by chemical means.

All fatty oils and fats contain smaller or larger amounts of free fatty acids in the natural state; in oils and fats of animal origin the proportion of free acids is normally very small; in vegetable oils and fats it is generally larger, an extreme case being that of palm oil, which may contain from 10 to 80 per cent. of free fatty acids. One of the principal objects of the refining processes to which vegetable oils and fats are submitted is the removal of these free acids by treatment with limited amounts of alkali; such processes are, however, only undertaken in the case of products in which the amounts of free acids are normally confined within reasonable limits.

All fatty oils and fats, especially those of vegetable origin, develop acidity on keeping, unless protected from light, air and moisture; this is, no doubt, to be ascribed to the action of hydrolysing enzymes which become active under certain conditions. The vegetable oils and fats are usually stored as reserve food material in seeds and fruits, and are accompanied by enzymes, the function of which is to convert them into soluble material which can be assimilated by the growing plant. This view is confirmed by the experience that if oil seeds or fruits are dried and pressed soon after they have been gathered, as, for example, in the case of the pre-war Cochin coconut oil, the resulting oil is of a much better quality than if they have to be kept for some time, often under adverse conditions, before the oil can be extracted; from this point of view, there is a great deal to be said in favour of pressing or extracting edible oils and fats from the seeds or fruits as near as possible to the locality where they are grown.

Rancidity is a term applied to various defects of taste which develop in oils and fats. It is not necessarily connected with a high content of free fatty acids, and cannot be satisfactorily detected or measured by chemical means, although several methods have been devised with this object in view. The development of rancidity is promoted by the action of air, light, and moisture, and probably also by enzymes derived from the original material or from micro-organisms, such as moulds. As is well known, the enzymes may remain active after separation from the organisms which produced them. The development of rancidity appears to be connected with processes of oxidation; it has been ascribed to the formation of aldehydes, ketones, acids, and various other substances; it is conceivable that such bodies as oenanthylic and pelargonic aldehydes and acids might arise from the oxidation of the unsaturated acids by cleavage at the point represented by the double bond. The process as a whole, however, does not lend itself to simple explanation. It may also be mentioned that oils and fats take up the odours and tastes of materials with which they are in contact, a property which has been made use of in perfumery, and which also forms the basis of the flavouring of butter and margarine fats. Taints in oils and fats, as well as in milk and butter, may also arise in this manner. Taints due to incomplete refining may sometimes also be described as rancidity. The whole question is of far greater importance with respect to edible fats, than fats required for other purposes.

Classification of the Fatty Oils and Fats.—Complex naturally occurring substances such as the fatty oils and fats cannot, of course, be submitted to any hard and fast rigid system of classification. The system adopted by Lewkowitsch, given below, is based partly on the origin of the product and partly on the drying power, *i.e.*, the power of absorbing oxygen with the formation of more or less viscous products. This important property depends on the proportion of unsaturated acid radicles present, and their degree of unsaturation; the combined effect of these chemical characteristics is accurately measured by iodine absorbing power, or, as it is generally called, the "iodine value" of the oil or fat. As pointed out by Lewkowitsch, the iodine value is the most convenient constant on which to base a system of classification, as on it depend to a large extent important physical properties, notably the consistence, melting and solidifying points.

The following scheme of classification should be compared with the table of constants and characteristics given on pp. 148 and 149:—

1.—*Liquid Fats.*

- (A) Vegetable oils.
  - (*a*) Drying, *e.g.*, linseed and hempseed oils.
  - (*b*) Semi-drying, *e.g.*, soya bean, cotton seed, rape seed, and sesame oils.
  - (*c*) Non-drying, *e.g.*, arachis, olive and castor oils.
- (B) Animal oils.
  - (1) Marine animal oils.
    - (*a*) Fish oils, *e.g.*, menhaden oil.
    - (*b*) Liver oils, *e.g.*, cod liver oil.
    - (*c*) Blubber oils, *e.g.*, seal and whale oils.
  - (2) Terrestrial animal oils, *e.g.*, sheep's foot and neat's foot oils.

2.—*Solid Fats.*

- (A) Vegetable fats, *e.g.*, palm oil, cacao butter, palm kernel and coconut oils.
- (B) Animal fats.
  - (*a*) Drying fats.
  - (*b*) Semi-drying fats.
  - (*c*) Non-drying fats, *e.g.*, beef tallow, mutton tallow, butter fat.

The various groups in this system of classification are, of course, by no means sharply defined, for all gradations in drying power and consistence are met with amongst the oils and fats.

THE ESTIMATION OF FATTY MATTER IN SEEDS, OILCAKE, ETC.

Vegetable oils and fats are obtained from seeds, fruits, etc., by submitting the disintegrated material to high pressure, either hot or cold, the finer, edible fats usually being expressed at the ordinary temperature (cold drawn). In recent years processes involving the extraction of fatty material by means of organic solvents have been introduced. Petroleum ether, carbon disulphide and chlorinated compounds of the carbon tetrachloride and trichloro-ethylene type have been used for this purpose. The exhausted material, known as oilcake, which usually contains from 6 to 16 per cent. of fat and varying proportions of nitrogenous matter, is largely used as fodder for cattle.

The method to be described is generally applicable for the determination of fatty matter in oleaginous seeds or fruits such as rape seed, linseed, soya beans, copra (*i.e.*, dried cocoanut endosperm), oilcake, etc.

If the material contains much water, it must first be dried at a gentle heat; seeds, etc., are first disintegrated by passing through a mincing machine, but if the material contains so much oil that loss takes place in this process, it is better first to break it up roughly by shredding with a sharp knife, with such precautions that any exuded oil may be washed into the extractor with the solvent. In dealing with copra, Bolton advises that the material should be cut radially so as to get a truly representative sample. It is generally agreed that petroleum ether is the best solvent to use, as ether will extract small amounts of non-fatty matter; for most purposes, petroleum ether boiling below 60° is suitable. The ordinary Soxhlet apparatus may be used, but the Bolton and Revis extractor described on p. 87 is recommended. The designers give the following directions for its use in addition to those already given: After a preliminary extraction lasting half to two hours, the extractor is removed and the solvent allowed to evaporate in a moderately warm place; when danger of spurting is passed, the contents are distributed over the walls of the tube by gentle tapping, and the tube is placed in the water oven for about half an hour. The contents are then transferred to a mortar with about 2 grams of clean dry sand which passes 60 mesh, and ground as finely as possible; the object is to break up the cell walls and liberate all the fat. The contents of the mortar are quantitatively returned to the extractor, the mortar being washed out with the solvent. The extraction is then continued for an hour to an hour and a half, after which the flask is removed and the solvent carefully distilled off on the water bath or a suitable electric heater, taking care in the latter case to avoid overheating towards the end. Care must be taken to avoid loss of fat by spurting on to the cork or the tube, for which reason it is well to use a flask with a somewhat long neck and to place it in an inclined position. The fat should be dried at a temperature not exceeding 105° till constant in weight. Unduly prolonged heating, or heating at too high a temperature, is liable to cause oxidation of oils and fats, especially of those with high iodine values and pronounced drying powers.

In dealing with substances such as flour and cocoa, it is usual to extract from 2 to 3 grams of the dried sample. One extraction will suffice unless the material cakes together, in which case it should be removed with the precautions described above, broken up, and further extracted. The fat in the flask is freed from the solvent by distillation and heating to 105° in an oven, and weighed.

The crude fatty matter obtained from a larger scale single extraction may be analysed for its content of free fatty acids, etc., as described below.

THE EXAMINATION OF THE FATTY OILS AND FATS.

Preparation of the Sample.—Before the sample is submitted to a chemical and physical investigation, it must be freed from foreign matter such as water, dissolved soaps, vegetable or animal tissues, etc. In most cases it will be sufficient to melt the sample, and, if water is seen to be present, allow it to stand in a warm place till separation has taken place, when the clear fat is passed through a dry filter. Dissolved mineral matter such as soap is detected by burning off a portion of the sample and examining the residue, if any, for metals; it may be removed by extracting the melted fat with dilute nitric acid and, after washing with warm water, proceeding as directed above. Foreign matter such as paraffin, rosin oil, etc., is not removed at this stage, and is only detected on closer examination.

The sample should always be thoroughly liquefied, at a gentle heat if necessary, and well mixed when portions of it are to be abstracted for analysis or physical determinations; it will be found that oils often deposit small quantities of solid matter at the ordinary temperature, which sink to the bottom of the vessel, while solid fats, when cooled slowly, first deposit their higher melting glycerides, so that the composition of the solidified mass may not be entirely uniform.

Estimation of Water in Fats.—This determination is of especial importance in the case of butter and margarine, in which the water and fats are mixed as emulsions (see p. 298).

According to the Sale of Butter Regulations of 1902, the percentage of water in butter is limited to 16 per cent. Similarly, margarine containing more than 16 per cent. of water would be held to be not genuine margarine but margarine and water.

For the determination of water in "acid oils," *i.e.*, mixtures of neutral oils and fatty acids obtained as a bye-product from refining processes, Bolton and Revis[1] recommend a special process.

THE PHYSICAL AND CHEMICAL CONSTANTS OF THE FATTY OILS AND FATS.

On pages 148-149 the physical and chemical constants of some of the commoner oils and fats are set out. As might be expected of natural products, variations occur between the constants of different samples of the same kind of material. Extreme variations are, however, exceptional, and for purposes of calculating the approximate compositions of mixtures, typical or average values are usually employed, the probability being that the results thus obtained will be near the truth.

PHYSICAL METHODS FOR EXAMINING FATTY OILS AND FATS.

These include determinations of specific gravity, melting point of the fat and of the fatty acids derived from it (titer test), and the refractive index. The last mentioned furnishes very valuable results. The titer test is generally of more value than the determination of the melting point of the fat itself. The specific gravity may furnish useful indications or corroborative evidence in some cases.

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[1] *Analyst* 1918, 159.

Specific Gravity.—This determination may be carried out by means of the Sprengel pyknometer or the ordinary specific gravity bottle; in most cases, however, sufficiently accurate results may be obtained by use of the Westphal balance which is described on p. 50. In case of oils which are completely liquid at the ordinary temperature, the specific gravity is taken at 15·5°, and in case of solid fats, at the temperature of boiling water. If it is only necessary to heat the fat a few degrees above 15·5° for complete liquefaction, the determination may be made at this temperature, and the result reduced to 15·5°. Allen gives the mean temperature correction for most common fatty oils and fats, with the exception of whale oil, as 0·00064 per degree Centigrade. The specific gravities of the common oils and fats are set out in the table on pp. 148 and 149.

Melting Point.—The methods commonly used for relatively pure organic substances cannot be applied to fats. Numerous methods have been designed for determining the melting points of fats, and as varying results are obtained according to the method used, it is not advisable to place too much reliance on indications afforded by comparison of results obtained with figures given in the literature. By the following method, which is used in several works' laboratories, a number of samples may be dealt with simultaneously.

Glass capillary tubes are made by drawing out thin-walled tube, the capillary part being as nearly as possible of 1 mm. bore and about 5 cm. long. The melted sample is introduced into the capillary end to a height of 1 cm. The fat in the tube is suddenly cooled by placing between two flat pieces of ice or in a freezing mixture made by mixing five parts of commercial hydrochloric acid with eight parts of powdered crystallised sodium sulphate immediately before use. The fat in the tubes should be kept for at least two hours on ice, or overnight in a cold place, if the freezing mixture has been used. Unless the fat is thoroughly set, the melting point observed will be too low. The tube is placed in a water bath so that the top of the fat column comes 1 cm. below the surface; while the water is kept well stirred, the temperature, as indicated by a delicate thermometer, is raised at the rate of about 1° per minute; the temperature at which the fat becomes sufficiently soft to be forced up the tube by hydrostatic pressure is taken as the melting point. If only one or two melting points are to be determined, the tubes may be attached to the thermometer bulb by a rubber band. If a number of tests are to be carried out simultaneously, the tubes may be held in position in shallow grooves in a long strip of wood by means of a wide rubber band which is wired down at several points throughout the length of the strip. The temperature of the water bath must be raised gradually and evenly, the heat being supplied not by a direct flame, but by immersing in a second water bath kept at a slightly higher temperature. The stirring should be carried out so as to cause a constant current of water to circulate past the melting point tubes and thermometer.

Some fats, especially coconut and palm-kernel fats, show perfectly sharp melting points by the above methods, duplicate tests agreeing within 0·2°, or at the most 0·3°. In the case of some animal fats or mixtures of these with oil, especially where the proportion of stearin to oil is small, the melting points observed are less sharp, the movement of the fat up the tube being sluggish.

The Titer Test.—By the titer test is understood the determination of the solidifying point of the insoluble fatty acids, together with the small amount of naturally occurring unsaponifiable matter, as obtained by the process now to be described. The procedure set out is that recommended by Lewkowitsch.

Saponify 100 grams of the oil or fat by boiling with 40 c.c. of aqueous potassium hydroxide solution of specific gravity 1·4, and 410 c.c. of alcohol, in a porcelain dish on a water bath, stirring continually until the soap becomes pasty. Dissolve the residual soap in 1,000 c.c. of water and boil until all the alcohol has been removed, replacing the water which has evaporated, from time to time. Decompose the soap solution by acidification with dilute sulphuric acid and when, by continued boiling, the fatty acids have been obtained as a clear layer floating on the aqueous liquid, draw off the latter by means of a syphon, and wash the fatty acids several times with hot distilled water until all acid, as tested for by methyl orange, has been removed. Place the dish with the fatty acids on the water bath until the latter are melted, and the water and impurities have settled out; after passing through a fluted, soft, thick filter paper in a heated funnel, they will be sufficiently dry for further examination. The actual determination may be carried out by the following method, due to Dalican and recommended by Lewkowitsch:—

The mixture of fatty acids, after standing overnight in a desiccator, is carefully melted in an air bath, and as much of it is poured into a test tube 16 cm. long and 3·5 cm. wide as will fill the tube somewhat more than half full. The tube is fitted by means of a cork into a wide mouth bottle, 10 cm. wide and 13 cm. high; an accurately standardised thermometer, graduated in tenths of a degree, from 0° to 60° C., having a mercury bulb, 3 cm. long and 6 mm. in diameter, is placed so that the bulb is in the centre of the mass of the fatty acids. When a few crystals appear at the bottom of the tube, the mass is stirred by giving the thermometer a rotatory movement, first three times from right to left and then three times from the left to right. The mass is then stirred continually with a quick circular movement of the thermometer without allowing it to touch the sides of the vessel, and taking care that all the solidified portions, as long as they form, are well stirred into the mass until it has become cloudy throughout. At this point the thermometer is carefully observed; at first the mercury will fall or remain stationary, after which it will suddenly rise some tenths of a degree, remain stationary for a short time and then fall again. The maximum temperature attained during this rise is the titer or solidifying point of the mixed fatty acids.

The above method gives very concordant results if attention be paid to detail, especially in the actual determination of the solidifying point.

The titer test is largely used for the commercial valuation of fats used in soap and candle making, notably tallow and palm oil. It is customary to stipulate that the solidifying point shall not lie below a certain value, say, 43·5° C. in the case of beef tallow, and to reject the material or make a deduction in the price paid for it if it fails to comply with this standard.

Reference to the table on p. 148 will show that the mixed fatty acids derived from various oils and fats may, in some cases, readily be distinguished from one another by means of the titer test; thus, compare the figures given for cotton seed oil and rape seed oil. This circumstance may be made use of in the examination of fatty oils and fats in mixtures with mineral oils, it being possible to separate the fatty acids from such mixtures, but not the fatty oils or fats themselves. This problem will be further discussed under the examination of lubricating oils. (Chapter V.)

Refractive Index.—The instruments generally used for the examination of oils and fats are the Abbé refractometer, which has a range of from 1·3 to 1·7, and the Butyrometer or butter refractometer, which has a range of from 1·42 to 1·49. The latter instrument is specially designed for examining oils and fats, and has a scale in arbitrary divisions the readings of which are generally quoted in the text-books and literature relating to the present subject. The relations between the Zeiss scale divisions and refractive indices are given in the following table :—

Scale degrees.	Refraction Indices.	Scale degrees.	Refraction Indices.
0	1·4220	60	1·4659
10	1·4300	70	1·4723
20	1·4377	80	1·4783
30	1·4452	90	1·4840
40	1·4524	100	1·4895
50	1·4593		

Both instruments have the following points in common. They consist essentially of a telescope and two dense glass prisms, between which a continuous film of the (clear) liquid under examination is introduced. The field will be found to be divided into light and dark portions (owing to total reflection phenomena), and the dividing line is made as sharp as possible by focussing the telescope. Both prisms are provided with jackets through which water at a definite constant temperature is circulated (see Fig. 13). It is desirable that all observers should take observations at the same temperature, *i.e.*, 40°. An accurate thermometer is placed so as to indicate the temperature of the water as it leaves the prisms, and this should have been constant for a few minutes before taking the readings. If the

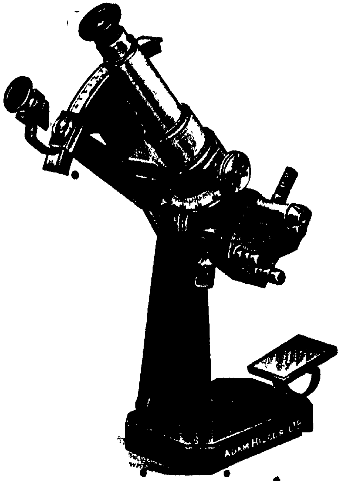

FIG. 12.—Abbé Refractometer.

temperature is not exactly 40°, a correction of 0·55 scale degree per degree C. may be applied. The writer finds that a good deal of time may be saved by making use of a chart described by Richmond (*Dairy Chemistry*, p. 350); he prefers to set it out to about four times the scale recommended by Richmond and to include only temperatures from, say, 38° to 42°, the central line representing 40°. The instruments should be handled by grasping the base and not the telescope. The prisms should always be left clean by wiping them carefully with soft, clean linen or muslin, using a little alcohol and ether if necessary, and the greatest care should be

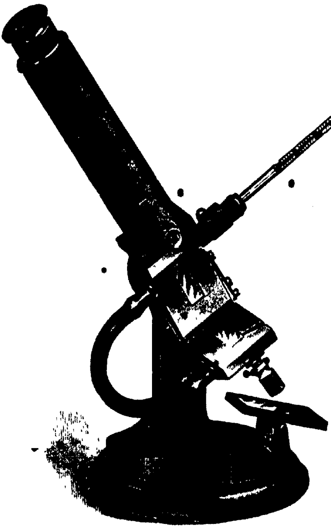

FIG. 13.—Butyro-Refractometer.

taken to protect them from abrasion. The same applies to the metal parts surrounding the prisms. From time to time, the adjustment of the instrument should be tested by means of the standard liquid or test piece supplied by the makers.

In the butter refractometer the position of the shadow line is observed on the scale inside the telescope; tenths of a scale division, corresponding to the fourth decimal place of the refractive index, are estimated by means of a micrometer screw. In the Abbé instrument cross lines in the telescope are made to coincide with the shadow line by rotating the arm connected with the prisms, and the reading is obtained from the circular scale, the fourth place of decimals being read by estimation. Both instruments can be used with daylight, the Abbé instrument being provided with a compensating arrangement to produce an achromatic field. In the butter refractometer the shadow line is sometimes not so sharp, but this defect may be overcome by using sodium light.

By the use of the refractometer a number of samples may be dealt with in a short time; the values obtained, some of which are given in the table on p. 148, are extremely useful for checking the purity of materials and for estimating adulterations. There is a certain parallelism between the refractive index and the degree of unsaturation, or the iodine value of the oil.

The Abbé refractometer is the more expensive instrument, but it can be applied to other work than the examination of oils and fats, *e.g.*, essential oils, strength of sugar, and other solutions, etc.[1]

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[1] Four papers have recently appeared on this subject, by J. C. Phillip, F. Stanley, F. Twyman and F. Simeon, and Hugh Main, Annie Homer and A. E. Berry, *J.S.C.I.*, 1919, 38, 139 T.

CHEMICAL METHODS FOR EXAMINING FATTY OILS AND FATS.

Under this heading will be described the determination of (*a*) the iodine value, (*b*) the saponification value, and (*c*) the Reichert-Meissl, Polenske-Kirschner values. These may be considered as being, within certain limits, constants for the individual fatty oils and fats, and may often be employed as a basis for their identification and approximate estimation in mixtures. In addition will be described the determination of (*d*) the acid value, and (*e*) the unsaponifiable matter. The former of these may vary with the age and previous history of the sample, and cannot be looked on as a constant to be used for the purposes of identification. The amount of unsaponifiable matter is normally small in most of the common fatty oils and fats, and its quantitative determination is generally of no great importance, except in cases where the presence of added foreign material is suspected. The qualitative examination of this constituent, also to be described below, is, however, of some importance in the differentiation of animal and vegetable fats, and especially in the detection of the latter in the former. (See "Phytosteryl Acetate Test.")

Iodine Value.—The iodine value expresses the number of parts by weight of iodine which can be absorbed by 100 parts of the fat. In Hübl's original process the fat was treated with iodine in an alcoholic solution containing mercuric chloride. In Wijs' process, which is described below, a solution of iodine monochloride in glacial acetic acid is used, the time required for absorption being considerably shorter than in Hübl's process. As the

Wijs' process is practically exclusively used nowadays, the alternative processes need not be described.[1]

In the determination a weighed quantity of the fat, dissolved in carbon tetrachloride, is treated at the ordinary temperature with a definite volume of the iodine monochloride solution, and an equal volume of the latter is set aside at the same time, under similar conditions, as a blank test. After the requisite time has elapsed the iodine monochloride is estimated in each case by titration with sodium thiosulphate solution. The difference in the number of c.c. required in the blank test and in the actual determination is calculated to express the percentage proportion of iodine absorbed by the fat.

The following solutions will be required:—

Iodine Monochloride Solution.—Pure glacial acetic acid is prepared by the writer as follows:—The glacial acid obtained from the dealers is treated with small quantities of powdered potassium permanganate until a permanent brown colour remains after standing for a few hours. It is then distilled from a flask with a side tube immersed in a heated oil bath. The distillate between 117° and 123° is collected separately and used; 99·9 per cent. acid, free from empyreumatic compounds, is obtained in this way. 10 grams of iodine trichloride (a 10-gram tube if obtainable) are dissolved in 200 to 300 c.c. of the acetic acid in a flask provided with a cork carrying a long glass tube, to exclude moisture, heating on a water bath. 12 grams of iodine are likewise dissolved in about 600 c.c. of acetic acid. The iodine solution is added to the iodine trichloride solution, and the progressive change in colour noted; the final change should be from an orange to a brown shade, and, if necessary, a little more iodine solution should be added to ensure that there is a slight amount of iodine in excess of that required to convert all the trichloride into monochloride. The solution is then made up to a litre with acetic acid, heated for twenty minutes on the water bath, and preserved in a well-stoppered bottle avoiding access of moisture at all times.

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[1] See Fahrion, *J.S.C.I.*, 1915, 34, 185.

Sodium Thiosulphate Solution.—24 grams of the crystallised salt are dissolved in water and made up to 1,000 c.c. This solution may be conveniently standardised by the following method, due to Volhardt; 3·8631 grams of pure potassium dichromate, free from the sodium salt, are dissolved in water and made up to 1,000 c.c. This solution should be standardised from time to time by titration against standard ferrous ammonium sulphate solution. 10 c.c. of a 10 per cent. solution of potassium iodide solution and 5 c.c. of hydrochloric acid are placed in a stoppered bottle and exactly 20 c.c. of the dichromate solution are run in. The resulting mixture now contains exactly 0·2 gram of free iodine, which may be titrated with the sodium thiosulphate solution, using starch as an indicator towards the end of the process. The iodine equivalent of the thiosulphate solution may then be calculated.

Starch Solution.—About 1 part of starch is stirred up in 100 parts of water, and the mixture heated to boiling.

Potassium Iodide Solution.—A 10 per cent. solution of the pure salt in water.

To determine the iodine value, the fat is weighed out in a small specimen tube which is then dropped into a narrow mouthed bottle of about 500 c.c. capacity, and furnished with a well-fitting stopper. The amount of fat to be taken varies with its power of absorbing iodine;

in the case of a drying oil, about 0·15 gram should be taken, but with a solid fat, having a low iodine value, such as coconut oil, the amount may be increased to about 1·5 gram. In any case, the amount of fat taken should be so regulated that the excess of iodine monochloride present after absorption is complete is more than half of the amount originally present. The fat is dissolved in 10 c.c. of carbon tetrachloride, and 25 c.c. of the iodine monochloride solution are run in from a pipette; in measuring out the solution for the blank test and for any other determinations which are to be made simultaneously, the same pipette should be used and allowed to empty itself in the same manner and drain for the same length of time in each case. The resulting mixture should be a clear solution; if turbid, more carbon tetrachloride should be added till the fat is completely dissolved. The bottle is now stoppered and allowed to stand in a dark place for the requisite time; loss of iodine by volatilisation may be guarded against by moistening the stopper with potassium iodide solution. The time required for complete absorption varies from half an hour in the case of solid fats to two hours in the case of drying oils.

The estimation of the excess of iodine chloride is carried out as follows: 20 c.c. of potassium iodide solution and about 300 c.c. of water are added; the mixture is then titrated with the standard sodium thiosulphate solution, shaking occasionally, in order that the iodine may be extracted from the lower layer of carbon tetrachloride; a little starch solution may be added towards the end of the titration, but not before the colour has been reduced to a very faint yellow. The difference in the number of c.c. of thiosulphate solution required in the blank test and in the actual determination is then calculated to express the percentage proportion of iodine absorbed by the fat. (The molecule of iodine chloride, ICl, is chemically equivalent to the molecule of iodine I_2, in the interactions involved in the process.)

The determination of the iodine value is of great importance as a method of characterising the fatty oils and fats. Owing to the comparatively low melting points of the glycerides of the unsaturated acids, there is a general tendency for oils and fats with high iodine values to possess lower melting points and softer consistencies than those with lower iodine values. This rule is, of course, sometimes modified by the nature of the saturated acid radicles, an extreme case in point being that of coconut oil, which contains an unusually large proportion of saturated acid radicles of comparatively low molecular weight, and therefore possesses a rather low melting point in spite of its exceptionally low iodine value.

As was pointed out above, the drying power of oils and fats is, generally speaking, directly proportionate to the iodine value. This property of absorbing oxygen on exposure to air at the ordinary temperature or on "blowing" with air at elevated temperatures, with the formation of more or less viscous products, finds extensive technical application. Thus linseed oil (note the high iodine value), especially after it has been treated with lead and manganese oxides at about 150°, readily dries to a tough skin when exposed to air in thin layers, and is on this account extensively used in the manufacture of paint and linoleum. Other oils, such as cotton seed and rape seed oils, are often blown with air at about 150° in order to increase their viscosity for use as lubricants when mixed with mineral oils.

The diminution of the iodine values of cotton seed or rape seed oils on treatment with a current of air at about 120°, may easily be demonstrated by a small laboratory experiment.

Examples of the application of the results of iodine value determinations in the identification of the oils and fats, and the qualitative and approximate quantitative analysis of mixtures, will be found below. (See p. 150 *et seq.*)

Saponification Value.—The saponification value expresses the number of milligrams of potassium hydroxide required for the complete saponification of 1 gram of the fat. It is thus, in the case of pure fats, inversely proportional to the mean molecular weight of the acid radicles present, more alkali being required to saponify a given weight of fat consisting of acid radicles of low molecular weight combined with the glycerol residue than an equal weight of fat containing acid radicles of higher molecular weight. On this account, the saponification value affords a means of identifying or detecting certain fatty oils and fats, and, at the same time, of detecting admixed unsaponifiable matter, such as paraffin, the presence of which will obviously tend to give an abnormally low value.

From the following description of the process for determining the saponification value it will be seen that some of the potash used in the determination will go to neutralise any free fatty acids which the fat may contain. If the amount of free fatty acids should be considerable (see below, under "Acid Value"), then the saponification value found should be corrected to give the true "Ester Value" of the fat, by subtracting the weight of free fatty acids from that of the fat taken and the weight of potash required for the neutralisation of the acids from the weight of the potash used up in the determination. Similarly, in the case of notable quantities of added unsaponifiable matter being present, the weight of the latter, if estimated, may be subtracted from the weight of the sample used in the determination in order to arrive at the saponification value of the fat present in the mixture. The actual details of these calculations will be obvious from what is said below on the calculation of the saponification and acid values.

For the determination of the saponification value the following solutions will be required :—

Alcoholic Caustic Potash Solution.—35 to 40 grams of potassium hydroxide, purified by alcohol, are dissolved in about 40 c.c. of water, and made up to 1,000 c.c. with 96 per cent. alcohol ; the latter should be tested before use by boiling a few c.c. with an equal bulk of concentrated caustic alkali solution, whereupon only a very faint yellow coloration should be produced. The admixture of the aqueous potash and the alcohol may be facilitated by shaking and warming. After standing for a day, the clear liquor is decanted or filtered from any sediment which may have been deposited, and preserved in a bottle furnished with a well-fitting rubber stopper, so that it will be kept out of contact with atmospheric carbon dioxide. The solution should only become light yellow on prolonged standing if the alcohol used in its preparation was sufficiently pure ; otherwise it may turn dark brown.

Standard Hydrochloric Acid of half Normal Strength.—In the determination 1·5 to 2 grams of the fat are weighed into a 200 c.c. resistance glass conical flask, and 25 c.c. of the alcoholic potash solution are added from a pipette.

At the same time, a blank test is started, 25 c.c. of the same solution being measured into a similar flask, from the same pipette, in exactly the same way; this portion is to be treated in exactly the same way as the portions containing fat, in order that errors owing to absorption of carbon dioxide, and other causes, may be eliminated. The flasks are fitted with simple tube condensers, about 12 inches long, by means of rubber stoppers, and the contents are kept gently boiling on a water bath, and carefully agitated from time to time in order to hasten the saponification. This part of the process will be complete when a clear, homogeneous liquid, free from particles of fat, has been obtained, the time usually taken being from twenty minutes to half an hour; if, however, the presence of appreciable amounts of unsaponifiable matter is suspected, the boiling may be prolonged somewhat, the mixture being frequently agitated in order to prevent the occlusion of unsaponified fat. The amount of potash left over from each of the saponifications, as well as that in the blank test, is now estimated by titrating the hot solutions with semi-normal hydrochloric acid, adding 1 c.c. of a 1 per cent. alcoholic solution of phenol phthalein in each case; if the saponification products should have set to a jelly, owing to loss of alcohol, a sufficient quantity of warm alcohol previously neutralised towards phenol phthalein, to give a clear solution, should be added.

From the difference in the titers in the blank test and in the actual test the number of milligrams of potash required to saponify 1 gram of the sample may be arrived at by a simple calculation.

Examples of the application of the results of saponification value determinations in the identification of oils and fats, and the analysis of mixtures, will be given below. (See p. 150 *et seq.*)

Besides furnishing a useful constant for the identification or detection of certain oils and fats, the saponification value determination often admits of the detection of unsaponifiable matter, which, if present in appreciable quantity, will be seen as an immiscible liquid or insoluble solid on diluting the neutralised saponification product with distilled water; any soap which may separate out at this stage will redissolve on warming. If, however, it is desired to examine the unsaponifiable matter, the saponification must be repeated on a larger scale. (See below, under "Unsaponifiable Matter.")

The Reichert-Meissl, Polenske, and Kirschner Values.—These methods include the estimation and examination of the volatile acids obtained from certain fats under standard conditions. Practically all the common oils and fats, with the exception of butter fat, coconut oil, and palm kernel oil, yield only small amounts of volatile acids in these processes, which are thus particularly adapted for the examination of these three fats, and their detection and estimation in mixtures.[1] Other methods of dealing with this problem have been devised, but those described are the best established, both as regards working details and published results. The following directions must be carefully followed:—

Five grams of the fat are weighed into a 300 c.c. flask (see Fig. 14) together with 20 grams of glycerine. To the mixture are added 2 c.c. of caustic soda solution, which has been made by dissolving pure soda in an equal weight of water, and allowing to settle, due precautions being taken against absorption of carbon dioxide. The

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[1] Bolton, Richmond and Revis, *Analyst*, 1911, 36, 333, and 1912, 37, 183.

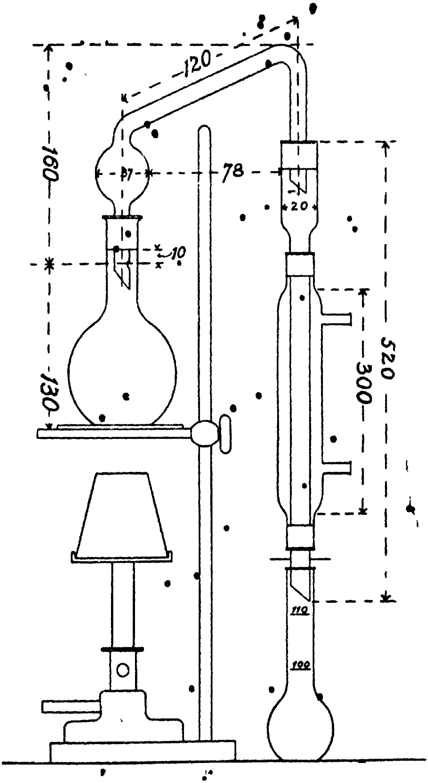

FIG. 14.—Polenske Apparatus.

flask is heated over a naked flame, shaking constantly until the fat has been saponified, *i.e.* when the liquid is clear and homogeneous; overheating must be avoided. After cooling for about two minutes, 190 c.c. of boiled distilled water are added and the soap dissolved. 0·1 gram of powdered pumice (sifted through butter muslin) is added, and then 40 c.c. of dilute sulphuric acid containing 25 c.c. of concentrated acid per litre, 35 c.c. of which should neutralise 2 c.c. of the strong soda solution. The flask is connected up without delay, as shown in the diagram, the apparatus being of the type and dimensions indicated. At first a small flame is used to melt the acids, the flask being shaken occasionally; then the flame is so adjusted that 110 c.c. of liquid will be distilled over in eighteen to twenty minutes; at the same time the flow of water must be so regulated that it leaves the condenser at a temperature of 18° to 20°. The flame is removed immediately the 110 c.c. have distilled over, and the flask with the distillate is stood in water at 10°, a small cylinder being put in its place to catch the drops from the condenser. After fifteen minutes the state of the insoluble acids is noted, the distillate is filtered, and 100 c.c. of the filtrate are titrated with decinormal baryta, using 0·2 c.c. of a half per cent. solution of phenol phthalein as indicator. The number of c.c. used plus 10 per cent., and less the value obtained in a blank test, is the Reichert-Meissl value. The blank test just alluded to is carried out with all the reagents, as just described, but without the fat; the glycerine and soda mixture should be heated exactly as in the actual test and not more.

Eighteen c.c. of water are poured through the condenser, caught in the cylinder, poured into the 110 c.c. flask, shaken and then poured on to the filter and rejected when filtered. Three portions of 20 c.c. of alcohol, neutralised to phenolphthalein, are then passed the same way, collected and titrated in the same way as the solution of the water soluble acids, the number of c.c. used, less the blank value, being the Polenske value.

The Kirschner value is determined as follows: To 100 c.c. of the neutralised solution of the water soluble acids obtained in the foregoing distillation process is added 0·5 gram of finely powdered silver sulphate, and the mixture is left for an hour, shaking occasionally. The precipitated silver soaps are then filtered off, and 100 c.c. of the filtrate are placed in a flask similar to that used in the previous distillation, with 35 c.c. of boiled distilled water, 10 c.c. of the dilute sulphuric acid, and a few inches of aluminium wire. 110 c.c. are distilled over exactly as described above, and 100 c.c. of the distillate are titrated with decinormal baryta solution. If the number of c.c. used, less the blank value $= N$, and the number of c.c. of alkali solution used in the Reichert-Meissl titration $= M$, then

$$\text{The Kirschner Value} = N\,\frac{1{\cdot}21\,(100 + M)}{100}.$$

The application of the above processes to the examination of butter and the estimation of butter, palm kernel and coconut oils in mixtures, is dealt with on pp. 160 and 166. It will be understood that accurate results can only be obtained by working under standard conditions. It may be explained that butter fat is unique in yielding a large proportion of soluble volatile acids of which the major portion is butyric acid. Usual limits are 26 to 33, and an arbitrary lower limit of 24 has been fixed for control purposes in England. Butters giving values below this limit should at any rate be regarded with suspicion, but it must also be remembered that the values may be influenced by the particular feeding and treatment of the cows. The Kirschner process is practically an estimation of butyric acid, silver butyrate being more soluble, and the silver salts of the higher soluble acids less soluble than silver sulphate. This process will probably prove superior to the Reichert-Meissl process for the analysis and the estimation of butter in mixtures. Palm kernel and coconut oils are distinguished by yielding high Polenske values depending on the presence of caprylic, capric, lauric, and myristic acids. Usual limits are 8 to 10·5 for palm kernel and 15 to 18 for coconut oil. The volatile insoluble acids from the former are solid, and from the latter liquid. Other similar though less common oils, such as Cohune and Babassu,[1] give appreciable Polenske values. Palm kernel oil usually gives Reichert-Meissl values from 4·5 to 6·5, and coconut oil from 6·5 to 8·5; as the corresponding Kirschner values are in the neighbourhood of 1 and 1·5 respectively, it will be seen that the estimation of butyric acid by the Kirschner process affords a sharper distinction between palm kernel and coconut oils on the one hand and butter fat on the other. Butter fat also gives small Polenske values ranging from 1·6 to 3·5, which should be roughly in proportion to the Reichert-Meissl values (Polenske's "new butter value"). Other edible oils and fats give values which usually lie between 0·1 and 0·3, so that the distinction here is quite sharp.

Acid Value.—The acid value is the number of milligrams of potassium hydroxide required to neutralise the free fatty acids contained in 1 gram of the oil or fat.

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[1] Bolton and Hewer, *Analyst*, 1917, 42, 35.

For the determination a convenient quantity, say, 20 grams of the oil or fat, are weighed off in a flask and treated with 50 c.c. of methylated spirit which has previously been neutralised with sodium hydroxide solution, using phenol phthalein as indicator. The mixture is heated to boiling and kept well shaken while it is quickly titrated with decinormal sodium hydroxide solution, in presence of phenol phthalein as indicator. On standing for a short while the pink colour indicating the end point of the titration will disappear, partly owing to absorption of carbon dioxide from the air, and partly owing to the saponification of the fat; the titration should, of course, not be continued on this account. If 20 grams of fat have been used, then the number of cubic centimetres of decinormal soda used, multiplied by 0·280, gives the acid value. It is sometimes the custom to calculate the percentage of free fatty acids expressed as oleic acid; this figure may be obtained by multiplying the acid value by 0·5. If it should be necessary to correct the saponification value to give the ester value of the fat, eliminating the effect of the free fatty acids (see p. 131), then the weight of free fatty acids, expressed as oleic acid, in the sample taken for the saponification value determination may be calculated and deducted in order to find the weight of fat actually taken, and the weight of potash required to neutralise the acids present deducted from the total amount of potash required to saponify the sample; the correction will, however, be unnecessary, unless the percentage of fatty acids be large. In the case of coconut and palm kernel oils, the free fatty acids are sometimes calculated to lauric acid, to find the percentage of which the acid value is multiplied by 0·357. This percentage can also be obtained by dividing the number of c.c. of soda by ten, if 20 grams of fat are taken. The Köttstorfer value expresses the number of cubic centimetres of normal sodium hydroxide solution required to neutralise the free fatty acids in 100 grams of fat.

As mentioned above, crude vegetable oils and fats almost invariably contain appreciable quantities of free fatty acids; thus, a sample of crude coconut oil will probably be found to have an acid value lying somewhere between 5 and 20, while a sample of the same fat which has been refined for edible purposes will show an acid value of less than 0·3. Oils and fats which are to be used as lubricants must not contain large proportions of free fatty acids, as these would act on the bearings to form metallic soaps which would exert a clogging effect. The acid values of such oils and fats should in any case lie well under 3, while in many cases it may be well to insist on an acid value not exceeding 1.

As regards edible fats, it is difficult to lay down any hard and fast limit for the permissible acid value, but experience shows that good vegetable oils and fats which have been refined by treatment with alkali and deodorised by steam nearly always have acid values under 0·3, sometimes considerably lower. In animal fats acid values up to 1·0 are generally not regarded as being too high; the natural free fatty acids are not removed from animal fats, and are much smaller in amount than in the vegetable products. In the cold drawn vegetable oils (*e.g.*, arachis or ground nut and olive oil may be so treated), which are usually of very good quality, a larger percentage of natural free fatty acids is not objectionable, acid values of at any rate up to 3·0 being quite permissible.

Separation and Examination of the Unsaponifiable Matter.—Under this heading will be described the method for isolating and examining the unsaponifiable matter which is a normal constituent of the fatty oils and fats. The detection and estimation of foreign unsaponifiable matter, such as paraffin wax, will be treated of later.

Salomon [1] found the unsaponifiable matter in a number of oils and fats to vary from 0·26 per cent. in the case of almond oil to 1·37 per cent. in the case of sesame oil. The characteristic substances are usually two complex alcohols; cholesterol in the case of animal fats, and phytosterol in the case of vegetable fats. These alcohols, which are generally examined in the form of their acetates (Bömer's phytosteryl acetate test), do not always constitute the whole of the unsaponifiable matter. Klostermann and Opitz [2] found the percentage of phytosterol to vary from 0·133 in olive oil to 0·549 in sesame oil (cf. the figures just quoted). Salomon (*loc. cit.*) found the melting points of samples of phytosteryl acetates from various vegetable fats to lie between 124° and 129°, and cholesteryl acetates from various animal fats to lie between 113° and 114°. Regarding the unsaponifiable matter of hardened oils, see p. 145. As a rule the natural unsaponifiable matter is only examined qualitatively, though a high content of such matter serves to characterise certain fats, such as mowrah fat and shea butter.

It may also be mentioned that the waxes, which consist of esters of higher insoluble alcohols, yield from

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[1] Chem. Zentrlbl. 1914, 1, 854, abs. *Analyst*, 1914, 39, 310.

[2] Zeitschr. für Nahr. u. Genussmittel, 1914, 28, 138, abs. *Analyst*, 1916, 41, 317.

35 to 55 per cent. of "unsaponifiable matter," owing to the fact that these alcohols, which take the place of the soluble glycerol in the oils and fats, are obtained together with the unsaponifiable matter proper, which in this case consists mainly of higher hydrocarbons. Salomon (*loc. cit.*) found in wool fat which belongs to the waxes, 51·6 per cent. of unsaponifiable matter of which 57 per cent. was not precipitated by digitonin. (See below.)

Phytosteryl Acetate Test.—This test depends on the fact that the melting point of phytosteryl acetate lies some 10° above that of cholesteryl acetate, and that the melting point of the latter is raised by the presence of the former. It is, therefore, possible to apply the test to the detection of the adulteration of animal fats such as butter fat or lard, with vegetable fats, as, for example, cotton seed oil, coconut oil, etc.

Various methods are available for the separation of the unsaponifiable matter. The oldest method, which need not be employed unless for any reason it is desired to separate the total unsaponifiable matter, consists in saponifying the fat, dissolving the soap in a sufficient quantity of water, extracting the solution with ether, washing the several ethereal extracts with water and evaporating the ether, or, alternatively, the dry soap, mixed with sand, may be extracted with petroleum ether. (See p. 144.)

The digitonin method, which was originated by Windaus, is the simplest and best method for the present purpose, if digitonin is available, which may not be the case for some time to come. It depends on the fact that cholesterol and phytosterol form insoluble compounds with this product of *digitalis*. The presence of paraffin wax or mineral oils does not interfere with the process; good results are obtained with "blown" or oxidised oils, and the acetates may be separated in a state of purity more readily than by the other methods. Marcusson and Schilling[1] operate as described below on the fat itself, but Klostermann and Opitz[2] recommend that the fatty acids should be used in the case of vegetable fats, the reason being that while cholesterol is present as such in the animal fats, most of the phytosterol is present in the form of esters in the vegetable fats, and must be liberated by saponification before it will combine with digitonin. Fifty grams of the melted fat or of the fatty acids obtained as described on p. 120 are shaken for fifteen minutes with 20 c.c. of a 1 per cent. solution of digitonin in 96 per cent. alcohol and allowed to stand in a warm place for several hours. The precipitate is separated as completely as possible by filtration and washed with ether till free from fatty matter; it is then heated for thirty minutes in a test-tube with 1·5 c.c. of acetic anhydride and the acetates which separate on cooling are examined as described below (p. 144).

Bolton and Revis recommend the following method by which practically all the unsaponifiable matter is separated:—Fifty grams of the filtered fat are boiled with two successive portions of 75 c.c. of 95 per cent. alcohol, which are poured off after cooling, mixed, and evaporated in a tared flask. The amount of fat present is roughly determined and saponified by two grams of caustic soda and 50 c.c. of alcohol per 5 grams. The soap is washed out into a porcelain basin and evaporated, stirring occasionally. When practically all the alcohol has been evaporated, twice as much sodium bicarbonate as caustic soda used is added together with two spoonfuls of fine sand; the mixture is stirred, evaporated to dryness, thoroughly ground and dried in the water oven, and then extracted for three to four hours (see p. 87) with petroleum ether, which must be perfectly free from residue. The unsaponifiable matter is obtained by evaporating the solvent.

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[1] Chem. Zeit. 1913, 37, 1001, abs. *Analyst*, 1913, 38, 458.

[2] Zeitschr. für Nahr. u. Genussmittel, 1914, 27, 713, abs. *Analyst*, 1914, 39, 310.

The small amount of unsaponifiable matter is acetylated as described under the digitonin method. Bolton and Revis acetylate with 2 to 3 c.c. of acetic anhydride in a well-stoppered one ounce bottle, tying down the stopper and heating in boiling water for fifteen to thirty minutes. They transfer the material in this and subsequent instances by dissolving in and rinsing the vessel (or filter) with small quantities of boiling absolute alcohol which are subsequently evaporated. 'If the cooled acetylation mixture is not clear, paraffin wax or mineral oil will be present, in which case the mixture must be treated according to Polenske's method, or the results will be vitiated. Lewkowitsch found it possible to detect paraffin wax amounting to 10 per cent. of the unsaponifiable matter as an oily drop floating on the acetic anhydride during acetylation. The acetic anhydride is distilled off by heating the bottle in an oil bath at 140° to 150°. Unless the unsaponifiable matter was separated by means of digitonin, the solution of the acetates in absolute alcohol may be coloured, in which case it should be heated in the bottle as before with a little finely powdered recently ignited animal charcoal till colourless. The acetates are repeatedly crystallised from the smallest possible quantity of 95 per cent. alcohol and the melting point (corrected, see p. 82) taken of each crop. The crystals may conveniently be filtered by suction, using a very small button as filter disc in a small funnel. The crystals are washed with a few drops of cold 95 per cent. alcohol and dried on a porous plate each time. Typical results for pure animal and vegetable oils are quoted on p. 141. The melting point of phytosteryl acetate from some vegetable oils may, however, be as high as 133°. If the digitonin method is not used, it may be necessary to recrystallise five or six times. The process need not be repeated when no appreciable rise is obtained between two successive crystallisations. With the digitonin method the maximum melting point is usually reached after the first or second crystallisation ; the following table shows results obtained by the writer with this method. It is generally agreed that if a melting point of 115° or 116° is not exceeded on repeated crystallisation, vegetable fats will be absent. If, however, a progressive and distinct increase is obtained above 114°, vegetable fats will be present.

Acetates from	Crude. Melting point °C.	Once crystallised. Melting point °C.	Twice crystallised. Melting point °C.
Margarine with 50 per cent. of animal fats . . .	123·5—125	127·5—128	—
Margarine with 20 per cent. of animal fats . . .	123·5—125	127 —127·5	—
Margarine of unknown composition	129 —131	131·5—132	—
Margarine of unknown composition	111 (about)	127 —129	127·5—129
Hardened whale oil, iodine value 6·7 .	111 —113	113 (sharp)	—
Cotton seed oil .	121 (about)	127 —128	127·5—128·5

The converse of the above process, *i.e.*, the detection of animal fats in the presence of vegetable fats, may be carried out by Windaus' method.[1] The method is a laborious one and requires very careful working.

THE IDENTIFICATION OF FATTY OILS AND FATS, AND THE ANALYSIS OF FATTY MIXTURES.

Examples of the application of the analytical methods described above, together with special tests by which certain oils and fats may be recognised or estimated, will be given under this heading. The analysis of fatty mixtures may in many cases be a matter of extreme difficulty, requiring considerable experience in this branch of analytical chemistry; the examples given below will only include comparatively simple cases of the detection of adulterations which have been known to occur in actual practice; only a limited number of the fatty oils and fats will be dealt with, while the analytical processes described in this chapter are by no means completely representative of the means available to the expert. In the table on p. 148 will be found a list of some of the more important fatty oils and fats, together with their physical and chemical constants, which may be determined by methods already described.

In the first place, the determination of the acid value and the saponification value or the unsaponifiable matter will show how far the sample consists of neutral glycerides. The specific gravity, which will generally only be determined in the case of the liquid oils, will not afford much definite information; in some cases abnormal values may give useful indications of adulterants to be looked for, or confirmation of conclusions arrived at by other means. The determination of the iodine value and refractive index, on the other hand, is of great use in the analysis of mixtures, owing to the considerable variations in this constant with the different oils and fats. As regards the saponification value, it will be noticed that the majority of the oils and fats have values lying in the neighbourhood of 190; rape, castor, and cod liver oils will be seen to be characterised by lower, and butter fat and coconut and palm kernel oils by higher, values. For reasons already pointed out, samples having high saponification values should be examined by the Reichert-Wollny process for volatile acids. The limitations of the use of the determination of the melting points of oils and fats for their identification has already been pointed out. Regarding the methods for examining the insoluble fatty acids, the titer test may often be of great value, while the determination of the iodine values of the fatty acids may be resorted to when the original sample contains large quantities of added unsaponifiable matter from which it cannot be separated.

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[1] Chem. Zeit., 1906, xxx, 1011. See also Lewkowitsch's treatise.

In addition to the chemical and physical methods, the taste and smell, especially on warming, of the sample, may afford useful indications. Even the non-expert may in some cases be guided by these methods, as, for example, in the detection of fish oils, especially if comparison be made with genuine samples. Matters may sometimes be simplified for the analyst by considerations of relative cost; thus, if a sample purporting to consist of a certain kind of oil or fat appears not to be genuine, it is obvious that the adulterant or substitute will only consist of a material which is cheaper at the ruling market prices.

CHEMICAL AND PHYSICAL CONSTANTS OF THE MORE WELL-KNOWN OILS AND FATS AND THEIR FATTY ACIDS.

(Chiefly based on Lewkowitsch, Bolton and Revis, and Fryer and Weston.)

	Specific Gravity $\frac{15^\circ}{15^\circ}$ C.	Refractive Index at 40° C.	Iodine Value.		Saponification Value.	Melting Point ° C.	Solidifying Point of Fatty Acids.	Melting Point of Fatty Acids.	Principal Uses.
			Fat.	Fatty Acids.[3]					
Linseed oil .	0·9135—0·937	72—75	175—200 **180**[1]	179—210	188—195 **193**[1]	—	19—21·6	17·0—22·5	Paint, varnish, linoleum and hardened oil.
Sunflower oil .	0·924 —0·927	60—63	122—133 **128**	110—134	190—194 **192**	—	17—18	22—24	Food.
Soya bean oil .	0·921 —0·927	61—63·5	122—135 **131·5**	—	190—193 **192**	—	23—25	27—29	Food and burning.
Maize (corn) oil	0·9215—0·927	59·5—61	111—125 **115**	113—125	188—193 **191**	—	14—16	18—20	Food, soap, and lubricant (blown).
Cotton seed oil[2]	0·918 —0·926	58—61	103—112 **110**	110—116	192—195 **193**	—	30—35	35—40	Food, soap, and lubricant (blown).
Cotton seed stearine .	0·919 —0·923	—	89—93	94·5	195	26—31	27—33	21—23	Food.
Sesame oil .	0·921 —0·924	58·5—60·5	103—112 **108**	109—112	188—193 **191**	—	18·5—23·5	25—32	Food, soap, and burning.
Rape oil .	0·913 —0·917	58—60	97—105 **101**	99—103	171—179 **175**	—	11·5—16·5	18—21	Food, lubricant (sometimes blown), and burning.
Arachis oil .	0·913 —0·919	55—57·5	85—95 **89**	96—103	190—196 **193**	—	28—31·5	27—31	Food, soap, and lubricant.
Olive oil .	0·915 —0·918	55—56	80—86 **85**	86—90	190—193 **191**	—	17—26	22—28	Food, soap, burning, and lubricant.
Castor oil .	0·960 —0·968	68—71	84—91 **86**	87—94	177—185 **183**	—	2·8—3·3	13	Medicine and lubricant.
Menhaden oil .	0·927 —0·932	68—72	141—171 **160**	—	188—193 **192**	—	27·5—28	21—25	Lubricant and burning.

Seal oil	0·917 —0·930	63·5—66	130—152	**140**	—	186—196	**193**	—	13·5—16	22—23	Lubricant, leather currying, and burning.
Whale oil	0·919 —0·933	56—60	110—130	**118**	—	188—194	**189**	—	22—24	27	Leather dressing, burning, soap, and hardened oil.
Japanese fish oil	0·917 —0·932	56—62	126—191	**192**	—	186—191·5	**191**	—	28—29	—	Lubricant and burning.
Cod liver oil[3]	0·922 —0·940	66—71·5	136—180	**161**	130—170	173—194	**186**	—	13 5—24	—	Medicine and leather currying.
Neat's foot oil.	0·915 —0·920	53·5—54·5	70—80	**78**	—	194—199		—	26·5—36·5	—	Lubricant and wool treatment.
Cocoa butter	0·950 —0·970	46—47·5	35—40	**37**	—	192—198	**193·5**	32—34	38—40	49—51	Food.
Palm oil.	0·921 —0·9245	41—45	51—57	**53**	—	198—202	**199·5**	27—40	36—45·5	47—50	Soap, candles, and lubricant.
Palm kernel oil	0·931 —0·938	36—38	15—20	**15**	—	242—250	**247**	27—29	20—22	25—28	Food and soap.
Coconut oil	0·923 —0·929	34·5—35·5	8—10	**9**	—	250—260	**257**	23·5—25	21—23	22—25	Food and soap.
Butter fat	0·936 —0·946	41—46	29—41	**36**	—	201—232	**225**	28—34	33—35·5	38—45	Food.
Beef fat (Jus.)	0·943 —0·953	46—48	38—44	**42**	—	193—200	**196**	42—50	43—45	43—47	Food and soap.
Lard	0·931 —0·938	49—52	50—66	**55**	—	194—197	**196**	32—48	34—39	37	Food, soap, and lubricant.
Beef stearine[4]	—	46—47·5	18—25	**20**	—	192—197	**195**	51—54	48—51	—	Food.
Beef oleo[4]	—	46·5—49	44·5—48	**46**	—	198—202	**199**	26—44	—	—	Food.
Sperm oil[5]	0·880	52	86		—	124		—	—	—	Lubricant.

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[1] These figures represent typical values for calculation.

[2] These values, especially the iodine value, depend largely on the amount of "cotton seed stearine" left in the oil.

[3] Medicinal.

[4] The harder and softer portions respectively of "Premier Jus," *i.e.*, refined beef tallow.

[5] This is a liquid wax.

The Detection and Estimation of Adulterants in Olive Oil.—Of the possible adulterants of olive oil, the following will be considered: cotton seed oil, sesame oil, arachis oil and rape seed oil.

Ex. 1. *Cotton Seed Oil in Olive Oil.*—On reference to the list of iodine values in the table, it will be noticed that olive oil possesses a lower iodine value than any of the adulterants named above. Although, in exceptional cases, perfectly genuine samples of olive oil have been known to give iodine values above 90, yet a sample showing an iodine value above the limits given in the table should be regarded with suspicion.

Cotton seed oil is best detected in presence of other oils and fats by *Gastaldi's modification of the Halphen test.*[1] Five c.c. of the oil are heated for four to five minutes over a naked flame in a test tube with five to six drops of a 1 per cent. solution of sulphur in carbon disulphide and three to four drops of pyridine, taking care that the temperature does not exceed 140°. A red colour is produced in the presence of cotton seed oil or cotton seed stearine. If the tube is heated in a boiling brine bath at 110°, the colour does not appear so soon, but danger of overheating is avoided; this method commends itself for routine practice. The original Halphen method, in which amyl alcohol is used instead of pyridine, is less delicate. As little as 0·25 per cent. of cotton seed oil may be detected by means of the modified test, by a yellowish rose colour. A faint reaction should therefore not be interpreted as a wilful adulteration in the absence of other evidence; sometimes slight unintentional adulterations may occur through the use of the same presses and vessels for successive batches of different oils in the refineries. It may be mentioned that cotton seed oil which has been heated above 200° does not respond to the above test.

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[1] Ann. Chim. Applic., 1914, 2, 203, abs. *Analyst*, 1915, 40, 15.

The only other oil which gives a coloration in the Halphen-Gastaldi test is kapok oil, an oil which resembles cotton seed oil in most respects. The two oils may be distinguished by means of *Milliau's modification of Becchi's test* as follows: Fifteen c.c. of the oil are saponified by heating in a dish on the water bath with a solution of 5 grams of caustic soda in 10 c.c. of water and 200 c.c. of alcohol. The soap is dissolved in 200 c.c. of boiling water, and the solution boiled to expel all the alcohol. The fatty acids are precipitated by a slight excess of dilute sulphuric acid, skimmed off, and then shaken with two successive portions of 15 c.c. of cold distilled water. The water is drained off, and the acids dried rapidly at 105°. Five c.c. of the acids are shaken with 5 c.c. of a 1 per cent. solution of silver nitrate in absolute alcohol. Cotton seed oil acids will develop a very slight brown colour, kapok oil acids a dark coffee colour. One per cent. of kapok oil may be detected in mixtures by this test.

If a positive reaction for cotton seed oil has been obtained, the extent of the adulteration may be approximately calculated from the iodine value of the sample as follows: Supposing the iodine value found to be 93. Then, taking the average iodine values of cotton seed and olive oils as 106 and 83 respectively, let x equal the percentage of cotton seed oil in the sample.

$$\text{Then } \frac{100-x}{100} 83 + \frac{106}{100} x = 93.$$

Whence x = from 40 to 45 per cent.

Similar indications would be afforded by the refractive index.

It will be observed that the difference between the saponification values of the two oils is not sufficiently great to afford a means for detecting the adulterant; this would also be the case if any of the possible adulterants mentioned above, with the exception of rape oil, were present. Confirmation of the presence of cotton seed oil might possibly be obtained from a somewhat high specific gravity and titer test, or melting point of the fatty acids, though the latter figures would not afford any basis for quantitative calculations.

Ex. 2. *Sesame Oil in Olive Oil.*—If the sample shows a higher iodine value than would be expected from genuine olive oil, and gives a negative test for cotton seed oil, then sesame oil should be tested for by the Baudouin test as follows: 10 c.c. of the oil are treated with 10 c.c. of concentrated hydrochloric acid and two drops of a 2 per cent. solution of furfuraldehyde, or 10 c.c. of concentrated hydrochloric acid in which 0·1 gram of powdered cane sugar has been dissolved; the mixture is shaken vigorously for one minute, and then allowed to stand to separate; in the presence of sesame oil the aqueous layer will be coloured a strong crimson.

This reaction allows of the detection of small quantities of sesame oil in mixtures, and is one of the most reliable of the colour reactions applied to oils and fats. For these reasons, the addition of sesame oil to margarine has been made compulsory in Germany and other countries, in order to provide a ready means of recognising this article of food. As some of the colouring matters used in margarine or butter produce a red coloration with concentrated hydrochloric acid, it is necessary, when testing for sesame oil in these, to wash the sample two or three times with the concentrated acid before applying the actual test, or to compare the colour with that produced by hydrochloric acid alone.

The proportion of sesame oil present in olive oil may be roughly calculated from the iodine value of the sample and the iodine values of sesame and olive oils, as shown in Ex. 1.

Ex. 3. *Arachis Oil*[1] *in Olive Oil.*—If the sample shows a somewhat high iodine value and gives negative Halphen and Baudouin tests, arachis oil should be tested for by Bellier's test, which, in common with the other tests described under this heading, is based on the fact that arachis oil is distinguished by containing appreciable amounts of glycerides of fatty acids which are less soluble than stearic and other acids generally obtained from oils and fats. Bellier's test furnishes indications which should be confirmed by examination of the sparingly soluble acids obtained by one of the other methods, having due regard to their several limitations which are mentioned below. In dealing with such mixtures of solid fats as do not admit of quantitative analysis (see p. 157), the writer has found Kreis and Roth's test useful.

Bellier's Test for Arachis Oil.—The modification given here is that adopted by Evers,[2] who has critically examined this and the following method in their various modifications. One c.c. of the oil is mixed in a flask with 5 c.c. of a solution made by dissolving 80 grams of pure potash in 80 c.c. of water and making up to a litre with 90 per cent. alcohol; it is saponified by heating on the water bath for four minutes under a reflux condenser. After adding 1·5 c.c. of acetic acid (one vol. glacial acid to two vols. water) and 50 c.c. of 70 per cent. alcohol (100 c.c. of 90 per cent. alcohol to 31 c.c. of water), the mixture is shaken and, if necessary, warmed till clear; it is then cooled to 15·5° and kept at this temperature for five minutes. A distinct turbidity will be obtained in the presence of as little as 5 per cent. of arachis oil. An approximate idea of the amount of arachis oil present may be got by immersing the flask in cold water, shaking continuously, and noting the temperature at which turbidity appears. It is best to warm just sufficiently to clear when this happens, and then to cool again, taking the second turbidity temperature as the one on which to base the estimation. Evers quotes the following table by Franz and Adler, for use in this connection :—

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[1] Known variously as "ground nut oil," "earth nut oil," "nut oil" or "pea nut oil."

[2] *Analyst*, 1912, 37, 487. References are given here to the literature of the subject.

Composition of Oil.	Turbidity Temperature.
Olive oil	11·8—14·3
,, with 5 per cent. arachis .	15·8—17·0
,, ,, 10 ,, ,, .	19·8
,, ,, 20 ,, ,, .	25·7
,, ,, 30 ,, ,, .	29·2
,, ,, 40 ,, ,, .	31·5
,, ,, 50 ,, ,, .	33·8
,, ,, 60 ,, ,, .	35·3
,, ,, 70 ,, ,, .	36·6
,, ,, 80 ,, ,, .	38·0
,, ,, 90 ,, ,, .	39·3
Arachis oil	40·0—40·8

The presence of solid animal or vegetable fats, other than coconut or palm kernel oils, will give rise to the formation of a precipitate which would mask the test for arachis oil.

Estimation of Arachis Oil.—The process described here is due to Evers (*loc. cit.*); it is a development of Renard's original process and Archbutt's modifications thereof. Five grams of the oil are saponified by heating for five minutes in a flask under a reflux condenser with 25 c.c. of the alcoholic potash solution used in the previous test. The hot soap solution is treated with 7·5 c.c. of the dilute acetic acid used in the previous test, and 100 c.c. of 70 per cent. alcohol containing one per cent. (by volume) of hydrochloric acid. The mixture is cooled to 12°—14° for one hour and then filtered; the precipitate is washed with the acid 70 per cent. alcohol, breaking it up occasionally with a loop of platinum wire, until the filtrate gives no turbidity with water. The washings must all be measured. The precipitate is dissolved in 25 c.c. to 70 c.c., according to its bulk, of hot 90 per cent. alcohol, and the solution is cooled to a fixed temperature between 15° and 20°, and, if crystals appear in any quantity, allowed to stand at this temperature for one to three hours, then filtered and washed first with a measured quantity of 90 per cent. alcohol (about half the amount used for the crystallisation), and second with 50 c.c. of 70 per cent. alcohol. The crystals are washed by means of warm ether into a weighed flask and weighed after distilling off the ether and drying at 100°.

The melting point of the crystals is taken in the usual way. The process depends on the fact that arachis oil yields some 4 to 5½ per cent. of acids melting from 71° to 73°, consisting chiefly of arachidic acid, $C_{20}H_{40}O_2$, and lignoceric acid, $C_{24}H_{48}O_2$, while other oils yield at the most only traces of these acids (see p. 109). If the melting point is below 71°, the material is recrystallised from 90 per cent. alcohol, noting the volume of solvent used in order that the necessary correction may be applied. If no crystals or only small quantities of crystals are obtained, sufficient water is added to dilute the alcohol to 70 per cent. strength (31 c.c. of water to 100 c.c. of 90 per cent. alcohol), the solution warmed to clear if necessary, and cooled to a definite temperature between 17° and 19°. In either case crystallisation is allowed to proceed at this temperature for an hour, after which the crystals are filtered off and washed with a measured quantity of 70 per cent. alcohol. If the melting point should still be below 71°, the material is recrystallised from a small quantity of 90 per cent. alcohol or again from 70 per cent. alcohol. The total quantities of these two solvents used throughout the whole process is noted, and the appropriate corrections made according to the following tables. The weight of acids multiplied by the appropriate factor given in the second table, due to Evers, will give the approximate percentage of arachis oil.

Weight of Acids obtained. (grams)	Grams of Acids dissolved by 100 c c. of 90 % Alcohol at (Archbutt)		
	15° C.	17·5° C.	20° C.
0·1 or less .	0·033	0·039	0·046
0·3 . . .	0·055	0·064	0·074
0·5 . . .	0·064	0·075	0·085
0·7 . . .	0·069	0·079	0·090
0·9 or more .	0·071	0·081	0·091

Weight of acids after correction for solubility in 90 % alcohol. (grams)	Grams of acids dissolved by 100 c.c. of 70 % alcohol.		
	M.P. 71°.	M.P. 72°.	M.P. 73°.
0·02 or less	0·006	0·005	0·004
0·02—0·05	0·007	0·006	0·005
0·05—0·08	0·009	0·007	0·005
0·08—0·10	0·011	0·007	0·006
0·10 or more	0·013	0·008	0·006
Factor for conversion of percentage of acids to arachis oil	17	20	22

In the presence of solid animal and vegetable fats, the test is not so satisfactory in its quantitative application, but may still be used qualitatively in cases where Bellier's test breaks down; according to Lewkowitsch, it is possible to estimate approximately arachis oil in lard by Renard's test. In such cases, however, it may be necessary to recrystallise repeatedly from 90 per cent. alcohol before an arachidic acid melting over 70° is obtained, while a negative result cannot always be taken as evidence of the absence of arachis oil.

Arachis oil is very frequently used as an adulterant in olive oil; as will be seen from the table on p. 148, the saponification value or specific gravity of the sample would not reveal the presence of this adulterant, while the difference between the iodine values of olive and arachis oils is not very great.

Ex. 4. ***Arachis Oil or Hardened Arachis Oil in Mixtures with Ordinary or other Hardened Oils.***—The following test due to Kreis and Roth[1] is useful for testing for arachidic acid in such mixtures containing hard fats as do not lend themselves to treatment by the processes described in the preceding example. The test itself, however, would not indicate whether the arachidic acid was obtained from hardened or natural arachis oil; in dealing with hardened oils the analyst is often in the unfortunate position of having to be content with very limited indications (see p. 168). The test resembles Renard's original test in that advantage is taken of the relative solubility of the lead salts of the unsaturated fatty acids and the insolubility of those of the saturated acids.

Twenty grams of the sample are saponified by heating with 40 c.c. of a 20 per cent. alcoholic solution of potash; 60 c.c. of 90 per cent. alcohol are added and sufficient 50 per cent. acetic acid to acidify the mixture (about 15 c.c.), which is then heated to the boiling point and treated with a hot solution of 1·5 grams of lead acetate in about 50 c.c. of 90 per cent. alcohol. After allowing to stand for twelve hours at the ordinary temperature, the lead soaps are separated and decomposed by heating with hydrochloric acid. The fatty acids thus obtained are crystallised three times from 90 per cent. alcohol, and the melting points of the crops are determined. If not less than 5 per cent. of natural or hardened arachis oil be present, the melting point will be above 70°.

Ex. 5, *Rape Oil in Olive or Arachis Oils.*—The presence of considerable quantities of rape oil in olive oil might be indicated by a somewhat high iodine value or refractive index, but in the case of arachis oil supposed to be adulterated with rape oil, no definite information could be obtained from the result of the determination of these constants. Bearing in mind the comparatively low saponification value of rape oil, it is evident that samples of olive or arachis oils adulterated with appreciable quantities of rape oil should show abnormally low saponification values. In this connection it may be mentioned that of the oils and fats given in the table on p. 148, which have low saponification values, rape oil is the only one which would be likely to occur as an adulterant of arachis or olive oils.

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[1] Zeitschr. für Untersuch. Nahr. u. Genussmittel, 1913, 25, 81, abs. *Analyst*, 1913, 38, 160.

The approximate proportion of rape oil present might be calculated from the saponification value of the sample as follows: Supposing that a suspected sample of arachis oil was found to have an iodine value of 97, and a saponification value of 187. Then, taking the mean saponification value of arachis oil as 193, and that of rape oil as 174·5, if x equals the percentage of rape oil present,

$$\frac{100 - x}{100} 193 + \frac{174{\cdot}5\,x}{100} = 187,$$

whence x = from 30 to 35 per cent.

In this case the determination of the specific gravity would be of little use, but a sample of arachis oil adulterated with rape oil might show an abnormally low titer test.

As rape oil has been shown to contain only very small amounts of arachidic acid, a determination of arachis oil as arachidic acid could be made as described above, in order to check the result obtained from the calculation based on the saponification value.

Rape oil may be detected and approximately estimated in mixtures by Tortelli and Fortini's method. (See Bolton and Revis, "Fatty Foods.")

Ex. 6. *Cotton Seed Oil in Rape Seed Oil.*—The presence of cotton seed oil as an adulterant in rape oil would be revealed by a higher saponification value than would be expected from pure rape oil, and might be confirmed by the Halphen test for cotton seed oil. The iodine value, refractive index, titer test, and specific gravity would all tend to be raised owing to the presence of the adulterant, though these figures, excepting, perhaps, the titer test, would not be noticeably influenced unless the amount of cotton seed oil present was large. The extent of the adulteration might be approximately calculated from the saponification value of the sample and the mean saponification values of cotton and rape oils, as described above in Ex. 6.

Ex. 7. *Arachis Oil, Sesame Oil, Cotton Seed Oil, Palm Kernel or Coconut Oil in Lard.*—The presence of any of the liquid oils would tend to raise the iodine value and the refractive index, whereas the presence of either of the two solid fats mentioned would tend to lower these figures. If one of the oils were added together with one of the solid fats in certain proportions, the iodine value or refractive index might obviously furnish no indication at all regarding the additions. Sesame and cotton seed oils could be detected by their characteristic reactions (see Exs. 1 and 2), while arachis oil, if suspected, should be tested for as described in Ex. 4. Coconut or palm kernel oils, if present in sufficient amount, would be indicated by a high saponification value, but better still by a definite Polenske (and Reichert) value. (See pp. 134-138.) Bolton, Richmond and Revis[1] have constructed curves from which the percentage of coconut or palm kernel oils may be deduced from the Polenske value. They give the following equations for these curves :—

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[1] *Analyst*, 1912, 37, 183.

$$x\,(\text{C.N.O.}) = 12{\cdot}3\,(P - 0{\cdot}45)^{0{\cdot}747,}$$
$$x\,(\text{P.K.O.}) = 16{\cdot}72\,(P - 0{\cdot}45)^{0{\cdot}806,}$$

where x = the percentage of coconut or palm kernel oil, and P = the Polenske value. These equations may easily be solved with the help of a logarithmic table. The writer has obtained very satisfactory results with the use of the curves, working with mixtures of known composition, the greatest error being ±3 per cent. The state of the insoluble acids will indicate which of the two fats is present. Burnett and Revis[1] have devised a method for examining the acids from the Polenske process in order to determine the relative proportion of palm kernel and coconut oils if both of these are present ; the method requires some practice with mixtures of known composition before it can be applied to unknown mixtures.

Confirmation of the presence of vegetable oils or fats could be obtained by means of the phytosteryl acetate test (see p. 142). This would be especially desirable if cotton seed oil were the suspected adulterant, for it has been shown that lard from hogs which have been fed on cotton seed cake may give a positive Halphen reaction for cotton seed oil. Paraffin wax has sometimes been added to defeat the objects of this test. (See p. 144 and Ex. 10.)

Ex. 8. *Arachis, Sesame, or Cotton Seed Oils in Cacao Butter.*—The adulteration of cacao butter, or chocolate fat, with the above-mentioned oils could be detected on the same lines as indicated in the previous example, excepting, of course, that the phytosteryl acetate test would in this case not be available, as cacao butter is itself a vegetable product. As cacao butter has a fairly low iodine value, the extent of the adulteration might be approximately calculated as indicated in Ex. I. The titer test and melting point of the fatty acids of cacao butter are fairly high, and would tend to be lowered through the presence of the oils in question. A determination of the melting point of the sample should also be made, as it is important that fats used in chocolate making should not melt at too low a temperature.

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[1] *Analyst*, 1913, 38, 255.

Ex. 9. *Fish or Blubber Oils in Rape Oil.*—Fish or blubber oils may often be detected in mixtures by their characteristic smell, which becomes more noticeable on warming. The presence of these adulterants would tend to raise both the saponification and the iodine values, especially the latter, on which quantitative calculation might be based as in Ex. I. Fish or marine animal oils, and other drying oils such as linseed oil, may be distinguished from, and detected in the presence of non-drying and semi-drying oils such as rape oil, cotton seed oil, arachis oil, etc., by means of the hexabromide test, which is based on the fact that the hexabromo (or octobromo) derivatives yielded by the first mentioned group are practically insoluble in ether and certain other solvents; the semi- and non-drying oils, on the other hand, are only capable of yielding lower bromine addition products which are soluble under the conditions of the test. (See p. 109.)

Halphen's modification of the hexabromide test is as follows: 0·5 c.c. of the oil are mixed with 10 c.c. of a mixture of 28 parts by volume of glacial acetic acid, 1 part of bromine and 4 parts of nitrobenzene, in a clean, dry test tube, the tube is closed and shaken, and the contents examined. The semi- or non-drying oils produce, at the most, only a slight turbidity, while the fish or marine animal oils, or other drying oils, produce a distinct precipitate which is not dissolved on the addition of 10 c.c. of methylated ether. Rape oil gives a turbid liquid separating into two layers, but on the addition of 10 c.c. of ether a clear liquid is formed.

The indications afforded are, of course, only of a qualitative nature, but the test would prove useful in deciding whether a sample of rape oil, showing an abnormally high iodine value, had been adulterated with a fish or other drying oil, or with larger quantities of a semi-drying oil having an iodine value higher than rape oil. Bolton and Revis state that as little as 5 per cent. of fish, marine animal or drying oil may be detected in rape oil, but that the test breaks down in the presence of animal fats such as beef tallow and lard, which produce a precipitate under the conditions of the test. Shea butter is stated to behave similarly to the animal fats.

Ex. 10. *The Detection of Paraffin Wax in Lard or Cacao Butter.*—The following method was devised by Polenske for the detection and estimation of paraffin wax in lard. It may also be applied to the detection of this adulterant in other fats.

As very small amounts of paraffin wax have sometimes been added in order to circumvent the phytosteryl acetate test, it may be necessary to work on not less than 100 grams of fat. In this connection it may be pointed out that if the digitonin method becomes universally available, this particular form of sophistication will probably disappear for reasons stated on p. 142. The fat is saponified, and the unsaponifiable matter extracted on the lines laid down on p. 143. If much paraffin wax is present, less fat may be taken, but the saponification treatment should be prolonged in order to ensure complete decomposition of the fat. The unsaponifiable matter is shaken with 1 c.c. of petroleum ether for twenty minutes at 15° to 16°, transferred to a small funnel plugged with cotton wool and washed with five portions of ½ c.c. of petroleum ether. The paraffin wax will be dissolved away together with some of the alcohols, but as more cholesterol is dissolved than phytosterol, the phytosteryl acetate test will be made sharper when applied to the residue as described above. The petroleum ether solution is evaporated and the residue transferred to a test tube, in which it is heated for an hour with 5 c.c. of pure concentrated sulphuric acid at 105°. After cooling, the acid is diluted and extracted with petroleum ether. The petroleum ether extracts, containing the paraffin wax unchanged, are evaporated in a tared flask and weighed after drying at 100°.

Butter and Margarine Fats.—The fats may be separated for analysis by filling the sample into an ordinary gas jar and placing it in a steam oven till separation has taken place. The fat is then filtered through a thick soft filter paper.

Genuineness of Butter Fat.—The most useful methods for examining butter fat are outlined under the Reichert-Meissl, Polenske, Kirschner processes. (See p. 134 *et seq.*) Some indication of the use of the Reichert-Meissl and Polenske values in this connection has already been given, but from what has been said it would appear that the relation between the Kirschner and the Polenske values afford the best means of judging butter fat. Bolton and Revis suggest the following values as standards :—

Kirschner Value.	Polenske Value.
20	1·6
22	2·1
24	2·6
26	3·2

A variation of 1·0 is allowed either way in the Polenske value corresponding to any particular Kirschner value, the addition of less than 5 per cent. of coconut oil causing the Polenske value to fall outside the limit. Palm kernel oil would naturally affect the values similarly, *i.e.*, lowering the Kirschner value and raising the Polenske value, but to a somewhat smaller extent in the case of the latter value. In the writer's experience, genuine butters give values falling within these limits. The subject is too complex to be pursued into detail here, but the following points may be noted : If a high Polenske value be found, the approximate amount of added palm kernel or cocoanut oil may be determined by calculating from the formulae given on p. 161, taking the Polenske value found and subtracting from it the average value which would be due to the butter present. The presence of vegetable fats may often be confirmed by the phytosteryl acetate test already described. It has sometimes been the practice to use the refractive index as a sorting test, but this is futile, as many margarine fat compositions give the same values as genuine butters. The information given by the Reichert-Meissl value is also inadequate when taken by itself, for a butter giving a high value may easily be adulterated with other edible fats and still give a value above the recognised limit of 24. In this respect the Kirschner value gives a sharper indication. Advantage may be taken of the Halphen and Baudouin reactions in testing for cotton seed and sesame oils. Examination of a thin film of the butter itself between the crossed Nicol prisms of a polarisation microscope may afford useful indications; butter should give a uniformly dark field except possibly for some isolated cubical crystals of salt, while if margarine is present, bright patches will appear. Renovated butter, *i.e.*, butter which has been melted and rechurned with fresh milk, will give the appearance of margarine. In difficult cases, the Avé-Lallemant method may prove useful for detecting adulterants where the indications given by the above methods are inconclusive. (See Bolton and Revis, " Fatty Foods.")

Margarine Fat.—An outline will be given here of some of the main methods available for the various types of margarine fat compositions. Margarine fats consist of mixtures of solid fats and liquid oils in proportions varying within certain limits. The solid fats may be (*a*) animal fats, *i.e.*, lard or beef fat; the beef fat used in margarine making, which contains all the glycerides naturally present, is known as " Premier Jus," and the softer portions, *i.e.*, jus from which stearine has been removed by fractional crystallisation, is known as " Oleo." (*b*) The vegetable fats coconut and palm kernel oils. (*c*) Hardened or hydrogenated fats, *i.e.*, oils which have been treated with hydrogen in presence of a catalyst whereby the unsaturated glycerides have been converted into stearine. The principal liquid oils used are arachis oil, cotton seed oil, and, more rarely, soya bean oil.

The Polenske value will at once show whether the above-mentioned vegetable fats are present or absent, and, if present, their percentage may be determined as explained on pp. 134 and 161. Butter will be indicated by a definite Kirschner value, and the percentage of butter fat in the mixture is given with fair accuracy by the following formula (Bolton, Richmond and Revis, *loc. cit.*, p. 160):

$$\text{Butter per cent.} = \frac{K - (0{\cdot}1P + 0{\cdot}24)}{0{\cdot}244}$$

where P = the Polenske value, and K = the Kirschner value.

The maximum amount of butter which may be added to margarine is fixed by law at 10 per cent. Microscopic examination of the crystalline structure of the fat may give useful indications. A drop of the melted fat may be placed on the slide, pressed out under a cover glass, and allowed to set slowly. The crystals may be seen very distinctly against a dark field if the polarisation microscope is used. Coconut and palm kernel oils crystallise as long needles radiating from common centres, while stearine from beef fat (or hardened oils) takes the form of feathery tufts. Lard crystallised from ether which has been allowed to evaporate spontaneously forms crystal with chisel-shaped edges which may be detected with some practice. Cotton seed and sesame oils may be detected by their characteristic reactions (see pp. 150 and 152), making due allowance for any colour which may be produced owing to the action of the acid on the colouring matter in the sesame oil test. Arachis oil, which is used in the best qualities, may be detected by the Kreis and Roth test (see Ex. 4). The iodine value or the refractive index, preferably the former, may be made use of in calculating the approximate percentages of the various ingredients on the lines laid down in Ex. 1, but unless the mixture is a fairly simple one, as in the case of a vegetable margarine, the problem may be very involved. The phytosteryl acetate test is not of much use in this connection, as vegetable oils are practically always present. It is unfortunate that the only chemical method available for the detection of cholesterol (see p. 146) is so laborious, for this would, at least in some cases, afford a means of deciding whether a margarine contained an animal fat or not. There is no certain method for detecting hardened oils, which have of recent years been used to some extent in margarine. The search for traces of nickel catalyst may be regarded as futile. Possibly some means may be elaborated on the lines of Moore's investigation on the iso-oleic acids of hardened oils.[1] The expert taster may, however, be able to detect these fats, which, in the light of Halliburton and Drummond's researches,[2] must be regarded as inferior substitutes for beef fat, owing to the absence of the fat soluble accessory growth substances.

In conclusion, it may be remarked concerning edible fats that palatability naturally plays an important part in their valuation, and that it is not susceptible of chemical measurement. (See pp. 111 and 140.)

From the above examples it may be seen how the analytical processes described in this chapter may be applied in the analysis of simple mixtures of the more well-known fatty oils and fats, or identifying unadulterated samples of the latter. The examples of adulteration given are all comparatively simple; in actual practice, however, problems may occur which will require for their solution more extensive investigations and greater experience in this branch of analytical chemistry than can be obtained from the present work. The student wishing to pursue the subject further is recommended to consult the works mentioned below.

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[1] *J.S.C.I.*, 1919, 38, 320 T.

[2] *J. Physiol.*, 1917, 51, 235.

REFERENCES.

"The Chemical Technology and Analysis of Oils, Fats and Waxes," by J. Lewkowitsch, 3 vols. 1913-1915. Macmillan & Co., London.

"The Laboratory Companion to Fats and Oils Industries," by J. Lewkowitsch. 1901. Macmillan & Co., London.

"Technologie der Fette und Oele," by Gustav Hefter. 3 vols. 1906 to 1910. J. Springer, Berlin.

"Analyse der Fette und Wachsarten," by Benedikt-Ulzer. 1908. J. Springer, Berlin.

"Handbuch der Chemie und Technologie der Oele und Fette," by Ubbelohde and Goldschmidt. 4 vols. 1908 to 1912. S. Hirzel, Leipzig.

"Laboratoriumsbuch für die Industrie der Oele und Fette," by J. Marcusson. 1911. W. Knapp, Halle.

"Chemisch-Technische Untersuchungsmethoden," Vol. III., by G. Lunge. 1911. J. Springer, Berlin.

"Allen's Commercial Organic Analysis" (revised edition), Vol. II. 1910. J. and A. Churchill, London.

"Fatty Foods," by Bolton and Revis. 1913. J. and A. Churchill, London.

"Edible Oils and Fats," by C. A. Mitchell. 1918. Longmans, Green, London.

"Technical Handbook of Oils, Fats and Waxes," by Fryer and Weston. 2 vols. 1917 and 1919. Cambridge University Press.

"Aids in the Commercial Analysis of Oils, Fats and their

Commercial Products," by G. F. Pickering. 1917. C. Griffin & Co., London.

" The Production and Treatment of Vegetable Oils," by T. W. Chalmers 1918. D van Nostrand Co., New York.

" The Hydrogenation of Oils," by Carleton Ellis. 1918. D. van Nostrand Co , New York.

" The Examination of Hydrocarbon Oils and Saponifiable Oils," by Holde, trans. by Mueller. 1915. John Wiley & Sons, New York.

" Andes' Drying Oils, Boiled Oil, and Solid and Liquid Driers," and " Vegetable Oils and Fats." English Editions by Stocks. 1917. Scott, Greenwood, London.

"Die Neueren Synthetischen Verfahren der Fettindustrie," by Klimont. 1916. Otto Spanner, Leipsic.

" Tables of refractive Indices," vol. 2 on oils and fats in preparation. Published by Adam Hilger Ltd , London.

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CHAPTER IV

SOAP

INTRODUCTORY

SOAP consists essentially of the sodium or potassium salts of the fatty acids, with or without admixture of the salts of rosin acids. They may be produced by the direct action of aqueous caustic alkali on the fat by agitating the mixture well and heating with steam, or, in the case of coconut and palm kernel oils, at the ordinary or slightly elevated temperatures. The fatty acids may also be prepared from the fats by various methods and then neutralised by alkali. Twitchell's reagent is largely used for hydrolysing fats; it is produced by allowing an excess of sulphuric acid to act on a solution of oleic acid in aromatic hydrocarbons, and is supposed to contain compounds of the type

$$C_6H_4SO_3H\ (C_{18}H_{35}O_2).$$

Lewkowitsch explains its action by the readiness with which it forms intimate emulsions with fat. Hydrolysis may also be accomplished by heating the fat with 3 per cent. of lime under a steam pressure of 120 lbs. per square inch. Finally, fats are hydrolysed by the action of certain plant enzymes, the lipolytic enzyme of the castor oil (Ricinus) seed having been employed on a large scale; this method has not been widely used.

The soda soaps are separated from the glycerol and the excess of water and alkali by adding common salt to the boiling mixture; being insoluble in brine, they are "salted out" in admixture with a relatively small proportion of water as a molten layer above the aqueous "lye." In making the finer grade soaps, more water may then be added, and the salting out process repeated. On the other hand, the soft potash soaps and the soda vegetable soaps made by the cold process cannot be salted out satisfactorily, and therefore contain the glycerol and excess of water and alkali. As explained on p. 138, coconut and palm kernel oils consist of the gylcerides of fatty acids of relatively low molecular weight; this explains why their soaps are more soluble in brine than other soaps; this property enables these soaps to be used with salt water, for which reason they are sometimes known as "marine or salt water soaps." Coconut and palm kernel oils may, however, be used in conjunction with other fats in the ordinary course of soap making.

The rosin, or colophony, which is used in conjunction with fats in the manufacture of cheaper soaps, consists of the residue which remains after distilling off the oil of turpentine and moisture from pine resin; it is chiefly composed of acids which dissolve in caustic alkali solution with the formation of soap-like products which generally differ from the true soaps produced from fats in possessing greater alkalinity, and being softer and darker in colour. Rosin or colophony should be distinguished from the rosin spirit or rosin oils which are produced from it by destructive distillation. (See Chapter V., p. 241.)

The fats most commonly used in soap making are tallow, palm oil, recovered grease, coconut oil, cottonseed oil, maize oil, sesame oil, palm kernel oil, various fish oils, castor oil, olive oil and lard, linseed oil and hardened oils (see p. 166). In the case of edible fats, it is usually the cheaper grades which are used in soap making.

The amounts of water incorporated with soaps may vary within wide limits. Materials, useful or otherwise, which may be added to soaps are mentioned below. The nature of the perfume added is a fairly important matter in the case of the finer toilet soaps, and this can very often only be determined by those who have an intimate experience of perfumery.

The cleansing action of soap depends, first, on the formation of small quantities of free caustic alkali owing to the hydrolytic action of water on the alkali salts of the fatty or rosin acids ; according to the principles of mass action, the greater the proportion of water to soap, the greater will be the extent of the hydrolysis, so that the concentration of free caustic alkali is to some extent automatically regulated and kept low ; further, the greater causticity of rosin soaps as compared with soaps formed from fats, may be explained by the fact that the rosin acids are weaker acids than the fatty acids, and, consequently, their sodium salts are hydrolysed to a greater extent in aqueous solution. The removal of dirt of a greasy nature, which cannot be effected by means of water alone, takes place as follows : A small portion of the fatty matter is saponified by the caustic alkali and removed as soluble soap ; the greater portion, however, is made into an emulsion with the alkaline liquid and mechanically removed with the lather.

The various soaps on the market may be broadly classified as follows :—

(1) *Toilet Soaps.*—These should consist as far as possible of the neutral sodium salts of the fatty acids together with moderate amounts of water. They should, naturally, contain no impurities or adulterants, and, above all, no free caustic alkali or alkaline carbonate. They are often made from lard or olive oil. The transparent toilet soaps which are produced by dissolving soap in alcohol and evaporating the clear solution, generally contain glycerol, and sometimes also notable amounts of sugar. Floating soaps contain air.

(2) *Laundry Soaps.*—These may contain more or less free alkali as carbonate or hydroxide. They are usually made from tallow, palm oil or rosin.

(3) *Commercial Soaps.*—The soft soaps, which are generally made from fish oils or vegetable drying oils, contain glycerol and excess of potash and water. The so-called hydrated soaps, which are produced from coconut or palm kernel oils by the cold saponification process, contain glycerol and excess of water ; they are also liable to contain unsaponified fat and free alkali, owing to the incompleteness of the saponification process. Marine or salt water soaps have been discussed on p. 172.

(4) *Medicated Soaps.*—These may contain phenol, cresols, naphthalene, and other coal tar products, or substances of a similar nature.

Besides unsaponified fat, free alkali, or free fatty acids, soaps may contain small amounts of chlorides, sulphates, silicates and other inorganic impurities derived from the materials used in their manufacture. According to Allen, the following additions are sometimes made to soap: oatmeal, bran, sawdust, fuller's earth, chalk, etc. Considerable quantities of sand or powdered quartz are used in scouring soaps. Sodium or potassium carbonates are added to scouring and commercial soaps in order to increase their detergent properties and also to facilitate lathering with hard waters. Petroleum or coal tar oils may also be added with a view to increasing the detergent properties. Iron compounds, ochre, ultramarine and other colouring matters are added to produce the effect of mottling. Sodium silicate, aluminate and borate may also be found in commercial soaps.

THE ANALYSIS OF SOAP.

Among the more important constituents to be determined, are water, total alkali, fatty and rosin acids, free alkali as carbonate and hydroxide, and combined alkali present as soap. The estimation of adulterants, or fillers and legitimate additions, such as alkaline carbonate silicate and phenol, will also be described. The following scheme for the analysis of soap, which will first be briefly summarised here, is, to some extent, based on that due to Allen, Leeds, and others, which is adopted in Allen's "Commercial Organic Analysis," 1911 edition.

Sampling.—The sample for analysis should, in the case of a solid soap, be taken from the interior of the bar or cake, avoiding the outer dried portions. Liquid soap should be allowed to stand in a warm place till thoroughly liquid, and mixed before sampling. The sample should be kept in a stoppered bottle and weighed between watch glasses in order to prevent loss of moisture during weighing.

The various operations which have been outlined above are described in detail below. Subsequently, the estimation of phenols and neutral hydrocarbons in soap will be described, and finally the analysis of phenolic disinfectants containing soap, such as lysol and creoline, will be dealt with.

(*a*) *Water.*—The following method is due to Watson Smith: 5 to 10 grams of the soap, reduced to fine shavings, are placed in a large porcelain crucible which is set in a sand bath, heated by a small Bunsen flame. The soap is continually stirred with a glass rod (weighed with the crucible) having a rough jagged end to facilitate the breaking up of the mass. The process is usually complete in twenty to thirty minutes; when, on removing the flame, a piece of plate glass placed over the dish no longer collects moisture, the heating may be discontinued, and the dish and contents, together with the glass rod, allowed to cool and weighed. The loss in weight represents water with possible traces of alcohol and essential oils. Burning of the soap must, of course, be avoided; this will, however, if it occurs, immediately be noticed by the characteristic odour produced. The results of this process are stated to be accurate to within 0·25 per cent., which is sufficient for technical purposes.

The following method given by the American Bureau of Standards [1] for the determination of matter volatile at 105° is more accurate than the above method. Two grams are dried for one hour at 60° in a porcelain dish three inches in diameter, 50 c.c. of absolute alcohol are then added, the liquid evaporated, and the residue dried for one hour at 60°, and then for two hours at 105°. (The soap should be finely shredded in order that it may dissolve completely in the alcohol and be deposited as a thin homogeneous layer after evaporation.)

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[1] U.S.A. Bureau of Standards Department of Commerce, Circular No. 62. This circular gives methods for the analysis of soap, and standards for the various qualities.

The following method, due to Fahrion,[1] is given as being the most convenient where great accuracy is not necessary. Two to four grams of soap are heated over a small flame in a dish with three times their weight of oleic acid, which has previously been heated to 120° for several hours. The mixture should be stirred with a glass rod, weighed with the dish, until the soap dissolves to a clear liquid. When all the water has been boiled off and the mixture starts fuming slightly, the dish and contents are cooled and weighed. The results are stated to be accurate to within 0·5 per cent.

It should be remembered that added volatile matter (see pp. 174 and 175) will also be driven off with the water; in ordinary cases, however, this will be negligible.

The U.S.A. Bureau of Standards [2] gives the following limiting percentages for matter volatile at 105° (*a*), and standard percentages of volatile matter on the basis of which the matter not volatile at 105° is to be paid for as soap (*b*). Two per cent. to be deducted from the weight of soap paid for, for every 1 per cent. in excess of (*b*). Milled Soaps. (Toilet.) (*a*) 15, (*b*) 10. White Floating Soap. (*a*) 34, (*b*) 28. Salt Water Soap. (*a*) and (*b*) 55. Special and Ordinary Laundry Soaps. (*a*) 34, (*b*) 28. Moisture not to exceed 20 per cent. Four-fifths of a pound of matter not volatile at 105° to represent one pound of soap. Chip Soap. (Suitable for use with soft water in high-grade laundry work.) (*a*) 15, (*b*) 10. Nine-tenths of a pound of non-volatile matter to be taken as one pound to be paid for.

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[1] Zeitschr. f. Angew. Chemie, 1906, xix. 385, abs. *Analyst*, 1906, 166.

[2] See footnote 1 above (p. 176).

As will be seen from the table of typical analyses of soap on p. 196, the percentage of water varies very considerably in the different grades of soap. The best toilet soaps may contain as little as 10 to 13 per cent. of water, while some of the inferior hydrated varieties will contain as much as 70 to 80 per cent. Soft soap usually contains about 35 to 45 per cent. of water, and a good yellow soap some 15 to 25 per cent.

(*b*) *Unsaponified Fat and Other Matter Soluble in Petroleum Ether.*—Dissolve 5 grams of the soap in alcohol, evaporate to a paste, mix with clean dry sand, dry first on the water bath and then at 105°, stirring occasionally, and extract with petroleum ether as described on p. 87. (If sodium bicarbonate is added, as directed on p. 144, the free fatty acids, if any, will be excluded from the petroleum ether extract.) The petroleum ether extract is evaporated to dryness in a tared flask, dried at 100° and weighed.

In addition to extraneous unsaponifiable matter, such as vaseline, coal tar products, etc., the residue thus obtained will contain any unsaponified fat or free fatty acids which may be present, together with traces of essential oils added as perfumes, and unsaponifiable matter occurring naturally in the fats. Unless, however, notable amounts of foreign material have been added to the soap, this residue should, in most cases, be very small. The estimation of phenols and neutral oils in soap is described in a subsequent portion of this chapter. In Allen's "Commercial Organic Analysis," 1911 edition, Vol. II., p. 425, will be found a systematic scheme for the examination of the petroleum ether soluble material from soap.

(c) *Fatty and Rosin Acids.*—If the soap does not contain any appreciable amount of petroleum ether soluble material, and the last operation has been omitted, the operation now to be described may be commenced, either with a fresh portion of the original sample or the residue from the water determination. The material, if previously treated with petroleum ether, is spread out and warmed gently so that the solvent may evaporate; it is exhausted with boiling water, and the aqueous solution filtered or decanted from any insoluble matter that may be present. Normal nitric acid is then added from a burette until no further precipitate is formed; about 10 to 20 c.c. of the acid are further added, and the total quantity used is noted. The precipitated acids are allowed to solidify, and the aqueous liquid is decanted off and preserved for further examination. The acids are re-melted and mixed with hot water, allowed to solidify and the water decanted off and added to the main portion; this process is repeated two or three times, after which the acids are filtered off and washed with cold water until the washings are no longer acid to methyl orange. The washings are added to the main portion of the aqueous liquid, which will contain the total alkali of the soap as nitrate; generally also small quantities of chloride and sulphate, soluble fatty acids if the soap is derived from coconut or palm kernel oils; glycerol in the case of soft soap or soap made by the cold process; and any sugar, glycerol, dextrin, gelatine or other soluble foreign matter which may have been added to the soap.

The funnel with the filter containing the insoluble acids is placed over a small weighed beaker in an air oven, and the whole is heated to about 100°. As the filter dries, the bulk of the melted acids will pass through it into the beaker below, where they may be weighed after cooling. The acids adhering to the filter and funnel are washed by means of petroleum ether, which is completely volatile below 80°, into a tared flask, dried at 100° after removal of the solvent by evaporation, and weighed, their weight being added to that of the main portion. The weight of the total fatty acids, multiplied by 0·97, gives the weight of the acid anhydrides (or radicles) existing in the soap.

If a weighed portion of the acids is dissolved in neutral alcohol and titrated with standard alcoholic potassium or sodium hydroxide solution, using phenol phthalein as indicator, the total combined alkali existing as soap proper may be found; this is calculated to Na_2O or K_2O. The latter may also be found by subtracting the free alkali from the total alkali, determined as described below. The soluble fatty acids in the aqueous liquid, if any, are determined as described under (*e*) and added to the main portion in the final statement of the results of the analysis.

The above method may be varied in several ways. Sulphuric acid may be used in place of nitric acid for precipitating the fatty acids, if sulphates are not to be determined as under (*f*). For a rough estimation, the cake of fatty acids obtained from a larger portion of the sample may be dried between filter paper and weighed; sometimes a known weight of beeswax is added to form a cake with the acids; this is necessary in dealing with liquid or semi-solid acids, as would be obtained from soft soaps.

Other methods are based on the extraction of the fatty acids from the aqueous liquid by means of ether, and weighing the dried residue obtained after evaporation of the solvent, either as acids or after conversion into soaps. A separating funnel may be used for the ether extraction, in which case the operation should be repeated twice with smaller portions of ether, and the united extracts should be washed with small portions of water to remove mineral acids. Several continental workers advocate the use of Huggenberg and Stadlinger's " Sapometer," [1] which consists of a special graduated tube with a side tap by which an aliquot portion of the ethereal solution of acids may be drawn off, thus avoiding the somewhat cumbersome procedure with the separating funnel.

Fendler and Frank,[2] discussing the various methods of treating the fatty acids for gravimetric determination, make the following recommendations : Coconut or palm kernel oil acids can only be correctly estimated (owing to their comparative volatility) by titrating them to neutrality against phenol phthalein in ether and alcohol (or alcohol) solution with alcoholic soda or potash, evaporating the residue and drying it at 103° to 105°. The acids from linseed oil soaps give high results owing to oxidation on drying (see p. 130) whether as acids or as soaps ; these acids, or their soaps (obtained as just described for palm kernel or coconut oil acids) should therefore be dried in an atmosphere of carbon dioxide.

The valuation of soaps is largely based on the percentage of fatty acids, calculated to anhydrides, as indicated above. The best toilet soaps will contain some 80 per cent. of fatty acids, good household soaps

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[1] Chem. Zeit., 1912, 36, 938, abs. *Analyst*, 1912, 38, 479.

[2] Zeit. Angew. Chem., 1909, 22, 252, abs. *Analyst*, 1909, 166.

60 to 65 per cent. of fatty or fatty and rosin acids, while the cheaper grades which contain filling material, sodium carbonate or silicate, sand, etc., and the soft and hydrated soaps, may contain as little as 10 to 20 per cent. of fatty or fatty and rosin acids.

The U.S.A. Bureau of Standards[1] specify that liquid soap should be a clear solution of vegetable oil potash (or potash and soda) soap containing not less than 20 per cent. of soap.

(*d*) *Total Alkali.*—The total aqueous liquid separated from the fatty and rosin acids, including the filtrate and washings obtained as described under (*c*), is titrated with semi-normal potassium or sodium hydroxide solution, using methyl orange as indicator. The difference between the number of cubic centimetres of normal nitric acid used for the liberation of the fatty acids as described under (*c*), and half the number of cubic centimetres of semi-normal alkali used in the last titration, will be the number of cubic centimetres of normal acid equivalent to the total alkali of the soap. This is calculated to Na_2O or K_2O, as the case may be.

(*e*) *Soluble Fatty Acids.*—This estimation is based on the fact that the fatty acids show an acid reaction towards phenol phthalein but not towards methyl orange; the latter indicator may therefore be used for the estimation of free mineral acid by titration, in presence of fatty acids which may then be estimated by adding phenol phthalein to the solution which has previously been rendered neutral towards methyl orange, and continuing the titration with caustic alkali solution until a permanent pink tint appears. The determination of the soluble fatty acids is accordingly carried out by adding phenol phthalein to the solution from the determination of the total alkali (as described under (*d*)), and titrating with deci-normal caustic alkali solution. The number of cubic centimetres required to produce a permanent pink tint is calculated to caprylic anhydride,

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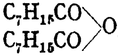
[1] See footnote, p. 176.



$$\begin{matrix} C_7H_{15}CO \\ C_7H_{15}CO \end{matrix} \Big\rangle O$$

, 2NaOH being equivalent to one molecule of the anhydride; the weight of the latter as Na_2O is added to the weight of the anhydrides of the insoluble acids already estimated as described under (*c*).

The presence of appreciable amounts of soluble fatty acids will indicate that the soap has been made from coconut or palm kernel oils.

(*f*) *Chlorides and Sulphates.*—These constituents may be determined in aliquot portions, say, one-fifth each, of the neutralised liquid obtained from operations (*d*) or (*e*). The chlorides are determined in the usual manner by titrating the faintly acid solution with deci-normal silver nitrate solution, using a few drops of potassium chromate solution as indicator, and continuing the titration until a permanent faint red-brown colour appears. The number of cubic centimetres of silver nitrate solution required is calculated to express the percentage of NaCl (or KCl) in the sample. Sulphates are determined by precipitation with barium chloride solution, in the usual manner, and the amount of barium sulphate weighed is calculated to express the percentage of Na_2SO_4 (or K_2SO_4) in the sample.

As will be seen from the table on p. 196, the better the quality of the soap, the smaller will be the percentage of sodium chloride and sulphate. The U.S.A. Bureau of Standards allow 1 per cent. each of Na_2SO_4 and NaCl in ordinary laundry soaps, and only 0·1 per cent. of Na_2SO_4 and 0·3 per cent. of NaCl in milled soaps.

(*g*) *Glycerol* (in absence of sugar).—The determination of this constituent is mainly of interest in the case of soft soaps, and the hydrated soaps made by the cold process. As was pointed out above, some toilet soaps may contain added glycerol.

For the estimation, an aliquot portion of the neutralised solution obtained from operations (*d*) or (*e*) may be used. The method to be described is due to Hehner, and depends on the quantitative oxidation of glycerol to carbon dioxide and water by means of potassium dichromate solution, the amount of dichromate used for the oxidation being measured by a titration process. The method is, of course, only applicable in the absence of other oxidisable matter such as sugar, which, if present, must be removed as described below under (*h*).

The following solutions will be required :—

Potassium dichromate solution, containing 74·56 grams of the pure salt and about 150 c.c. of concentrated sulphuric acid per litre.

Ferrous ammonium sulphate solution, containing 240 grams of $FeSO_4(NH_4)_2SO_4 6H_2O$ and 50 c.c. of concentrated sulphuric acid per litre.

Potassium dichromate solution of exactly one-tenth the strength of the other solution of the same salt. The stronger dichromate solution, 1 c.c. of which should be equivalent to 0·01 gram of glycerol, is titrated against the ferrous ammonium sulphate solution.

The solution in which the glycerol is to be estimated is made up to about 250 c.c. and transferred to a beaker which has been cleaned with potassium dichromate and sulphuric acid, in order to remove all traces of oxidisable matter. 50 c.c. of the stronger dichromate solution are then added, and the beaker is covered with a clock glass and heated for two hours in boiling water. At the end of this time an excess of the ferrous ammonium sulphate solution is added, so that the whole of the remaining potassium dichromate will be reduced to chromic sulphate, and a convenient amount of the ferrous salt left over for titration with the weaker dichromate solution. The approximate quantities of the various solutions required may be calculated from the following equation, representing the interaction between the dichromate and the ferrous salt in solution.

$$K_2Cr_2O_7 + 6\,FeSO_4 + 7\,H_2SO_4 = K_2SO_4 + Cr_2(SO_4)_3 + 3\,Fe_2\,(SO_4)_3 + 7\,H_2O.$$

The titration with potassium dichromate solution is carried out, using a weak solution of potassium ferricyanide as an outside indicator, according to the well-known method described in text books of quantitative inorganic analysis. The glycerol is quantitatively oxidised to carbon dioxide and water by the potassium dichromate, which is reduced to chromic sulphate as indicated by the equation given above. From these data the amount of glycerol present in the original solution may be calculated.

(*h*) *Sugar.*—If cane sugar, glucose, invert sugar, or dextrin are present, they will interfere with the estimation of glycerol by the above method. They may be tested for in the solutions obtained from operations (*d*) or (*e*) by warming with Fehling's solution, cane sugar or dextrin giving a reaction after the solution has been heated for fifteen minutes on the water bath with about 1 per cent. of hydrochloric acid. It may be pointed out that glycerol has a faint reducing action on Fehling's solution on prolonged heating.

Cane sugar has been added to toilet soaps, especially transparent soaps; in some cases as much as 20 to 30 per cent. of cane sugar have been found. Sugars of any kind should be absent from good soaps, as they can only be regarded as "fillers." The U.S.A. Bureau of Standards exclude sugar in their specifications.

(*i*) *Free Alkali or Fatty Acids.*—Five to ten grams of the original sample are dissolved in 200 c.c. of hot neutral alcohol in a flask, loosely corked to prevent absorption of moisture and carbon dioxide from the air. If the soap contains much water, it should first be partially dried in an atmosphere free from carbon dioxide. The hot solution is filtered rapidly through a weighed Gooch crucible to prevent undue exposure of the solution to air, care being taken that none of the soap jelly separates out on the filter. The filter is washed with absolute alcohol, and the total filtrate and washings titrated in presence of phenol phthalein with deci-normal acid or alkali, according to its reaction. If alkaline, the amount of standard acid required will give the percentage of free caustic alkali in the soap; if acid, the amount of alkali required is calculated to oleic acid ($C_{18}H_{34}O_2$) and returned as free fatty acids.

By titrating the neutralised alcoholic solution with acid, using this time methyl orange as indicator, the alkali existing in the soap as sodium salts of fatty or rosin acids may be found. (See also (*d*).)

The above method has recently been criticised by several workers. Other methods have been based on the precipitation of the fatty acids and carbonate as barium salts, thus Kling, Genin and Florentin [1] give the following method for determining caustic alkali in soaps (or commercial soda), precipitating in 50 per cent. alcohol owing to the fact that barium borate and silicate are appreciably soluble in water, and therefore likely to be estimated as caustic alkali if present. Fifty c.c. of a 1 per cent. solution of the soap (or soda) are treated with a 10 per cent. solution of barium chloride, using a slight excess above that required for complete precipitation. The mixture is treated with its own volume of 95 per cent. alcohol, allowed to stand for some time, and filtered; an aliquot portion of the filtrate is titrated at the boiling point with twentieth normal sulphuric acid, using turmeric as indicator. Reasonable precautions should, of course, be taken to prevent undue exposure to the air in order to avoid the absorption of carbon dioxide.

It will readily be understood that the determination of free caustic alkali is an operation of some importance in soap analysis, owing to the unpleasant effect of caustic soap on the skin, and the destructive effect of an excessively caustic soap on textiles. The U.S.A. Bureau of Standards [2] lays down maxima of 0·1 per cent. of free alkali as NaOH for toilet soaps, 0·5 as NaOH for laundry and salt water soaps, and 0·05 as KOH for liquid soaps.

(*j*) *Alkaline Carbonate, Silicate, etc.*—The residue insoluble in alcohol, left on the filter contains any carbonate, silicate, borate, aluminate, etc., together with other insoluble material such as starch, sawdust, sand, pumice, chalk, insoluble colouring matter, etc., which may have been present in the soap. The alkaline carbonate, silicate, borate or aluminate is separated from the total residue insoluble in alcohol by extracting with cold water and filtering. One half of the filtrate may then be titrated with deci-normal sulphuric acid in presence of methyl orange as indicator, in order to find the amount of soda present, while the other half may be evaporated to dryness and the nature of the alkaline material determined by examining the residue by the usual methods. For the determination of boric acid, see p. 414.

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[1] Bull. Soc. Chim., 1914, 15, 200, abs. *Analyst*, 1914, 224.

[2] See footnote, p. 176.

In a good toilet soap, the amount of alkaline carbonate should not exceed ½ per cent. In laundry, scouring and marine soaps, the addition of larger amounts of alkaline carbonate or silicate may be quite permissible.

The U.S.A. Bureau of Standards lays down the following maxima for the percentages of alkali as alkaline salts (Na_2CO_3): Toilet soaps, 0·3, not more than half of which shall be silicate; white floating soaps, 0·5; liquid soaps, 0·3 (as K_2CO_3); special laundry soap (for use with soft water), 1; ordinary laundry soaps (for use with moderately hard water), not less than 2 or more than 6, of which not more than half shall consist of sodium silicate; chip soaps (for use with soft water in high-grade laundry work), 0·5. The variation of the permissible amount of alkaline salts, according to the nature of the water, will readily be understood.

(*k*) *Insoluble Residue from* (*j*).—The residue left on the filter after extracting the alkaline material with water, may be dried, weighed and further examined. The substances mentioned above (p. 175) will readily be recognised by the usual tests, as well as their appearance. Microscopic examination may be useful here in recognising, for example, starch (see p. 305), kieselguhr (diatomite), fuller's earth, sawdust, etc. The U.S.A. Bureau of Standards limit for insoluble matter is 0·5 per cent. for ordinary laundry soap, and 0·1 per cent. for other soaps.

Total Alkali and Nature of the Same.—By titrating an aqueous solution of a known weight of the soap with standard acid, using methyl orange as indicator, the total alkali present in the soap may be found. This should agree with the sum of the determinations of alkali as (1) free hydroxide, (2) free carbonate, or silicate borate, etc., and (3) combined as soap with fatty or rosin acids.

If it is desired to determine the nature of the alkali, *i.e.*, whether soda or potash or a mixture of these, the alcoholic soap solution neutralised to methyl orange from operation (*j*) is treated with baryta solution until alkaline to phenol phthalein, and barium chloride solution is added as long as precipitation occurs. After filtering, the solution is evaporated to dryness, and the residue examined qualitatively or quantitatively for sodium or potassium by any of the usual methods described in the text-books on qualitative and quantitative inorganic analysis.

(*l*) *Detection and Estimation of Rosin Acids.*—Rosin acids may be detected by the Liebermann Storch reaction as follows: A small portion of the acids, precipitated and separated as described under (*c*) is dissolved in acetic anhydride at a gentle heat, and the solution allowed to cool. Sulphuric acid of specific gravity 1·53, prepared by adding 34·7 of the concentrated acid to 37·5 c.c. of water, is very carefully added when, in the presence of rosin acids, a reddish violet coloration will be developed. If the acid is not added with sufficient care, the mixture will become too warm, with the result that the coloration will not be observed, a brownish-yellow coloration being developed at once. Lewkowitsch points out that cholesterol gives a similar reaction to that obtained with rosin acids, and recommends that if the presence of this substance is suspected, it should be removed by extracting the aqueous soap solution with ether before liberating the acids for the test. As has been pointed out in the previous chapter on the fatty oils and fats, cholesterol is the principal unsaponifiable constituent of the animal fats. Wool fat contains much cholesterol.

For the estimation, the mixture of fatty and rosin acids isolated as described under (*c*), may be used, or a larger quantity may be prepared (see (*m*)). The method of Twitchell depends on the fact that while the fatty acids are esterified on treatment with hydrogen chloride in alcoholic solution, the rosin acids are unchanged, and may be estimated by titration with standard alkali solution in presence of phenol phthalein as indicator.

Two to three grams of the mixed fatty and rosin acids are dissolved in ten times their volume of alcohol, in a flask, and dry hydrogen chloride gas is bubbled through the solution until no further absorption takes place. The process will be complete in about forty-five minutes, when the ethyl esters of the fatty acids will have separated as an oily layer floating on the alcohol. The liquid is now diluted with five times its volume of water and boiled till the acid solution is clear, the esters containing the rosin acids in solution floating on the top. A little pumice or porous tile may be used to prevent bumping. The whole is transferred to a separating funnel by means of ether, and the ethereal layer is washed with water till all the mineral acid has been extracted from it; this will be accomplished when the aqueous layer no longer reacts acid towards methyl orange, or gives a precipitate or opalescence with silver nitrate solution in presence of nitric acid. The ethereal layer is now transferred to a flask, 50 c.c. of alcohol, neutralised towards phenol phthalein, are added, and the mixture is titrated with standard sodium hydroxide solution, using phenol phthalein as indicator. The titration should not be unduly prolonged, or partial hydrolysis of the esters by excess of alkali may take place. From the number of cubic centimetres of alkali solution required for neutralisation, the amount of the rosin acids may be calculated, assuming their combining weight to be 346.

Wolff and Scholtze[1] consider Twitchell's method inaccurate and recommend in its place the following method by which the fatty acids are esterified by methyl alcohol in presence of sulphuric acid. The method prescribed by the U.S.A. Bureau of Standards is practically identical with the exceptions that ethyl alcohol is used instead of methyl alcohol, and that the mixture is boiled for four instead of two minutes.

Two to five grams of the mixed fatty and rosin acids are dissolved in 10 to 20 c.c. of absolute methyl alcohol, and 5 to 10 c.c. of a solution of one part of sulphuric acid in four parts of methyl alcohol are added; the mixture is boiled for two minutes under a reflux condenser, mixed with 5 to 10 times its volume of 10 per cent. sodium chloride solution, and extracted several times with ether. The united ethereal extracts are washed with sodium chloride solution, alcohol is added, and the rosin acids are titrated with half normal alcoholic potash. Wolff and Scholtze make a deduction of

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[1] Chem. Zeit., 1914, 38, 369 and 382, abs. *Analyst*, 1914, 228.

1½ per cent. from the percentage of rosin acids found to allow for the fatty acids that have escaped esterification.

As mentioned above, rosin should only be present in the cheaper grades of soap; a good laundry soap should only contain about 20 per cent. of rosin acids. The U.S.A. Bureau ot Standards specifications allow 15 per cent. of rosin in special laundry soap, and 25 per cent. in ordinary laundry soap.

(*m*) *Nature of the Fatty Acids.*—The following methods are given by the U.S.A. Bureau of Standards (footnote, p. 176), for the separation of the fatty or fatty and rosin acids: *Toilet, Floating or Salt Water Soaps* are dealt with by treating a solution of 50 grams of the sample in 300 c.c. of hot water with 150 c.c. of approximately twice normal sulphuric acid, cooling and shaking out with 120 c.c. of ether; the ethereal extract is washed with strong sodium chloride solution until mineral acid (tested for in the filtrate by methyl orange) has been eliminated, shaken with 20 to 30 grams of anhydrous sodium sulphate and allowed to stand till clear, and evaporated in a current of dry air below 50°. *Laundry Soaps* are dealt with by heating a solution of 50 grams of the soap in 500 c.c. of hot water with 100 c.c. of 30 per cent. sulphuric acid and washing the resulting clear layer of fatty and rosin acids with hot water, filtering through a hot-water funnel and drying for twenty minutes at 100°.

The most important constants to be determined on the fatty acids are the solidifying point (see pp. 120 and 193) and the neutralisation value (see p. 193), which is worked out on the same basis as the acid and saponification values of fats (see pp. 138 and 131), and is mainly influenced by the presence of the acids of comparatively low molecular weight derived from coconut and palm kernel oils, which will also be indicated by the operation described under (*e*). Fryer [1] recommends the application of the standard Polenske process (see p. 134) for the determination of the acids of coconut and palm kernel oils, saponifying and treating 5 grams of the acids in the same manner as recommended for fats. He finds values of 17·3 and 10·5 for the insoluble acids of coconut and palm kernel soaps respectively.

In the majority of cases an exact determination of the origin of the fatty acids may be impracticable, especially when matters are complicated by the presence of rosin or the possible presence of acids derived from hardened oils. By the methods given above, however, a soap may be classified to a certain extent. The following requirements under the U.S.A. Bureau of Standards specification will give some idea of the results to be expected from various kinds of soap :—

	Solidifying Point of Acids. (Titer Test.)	Neutralisation Value of Acids.
Milled soaps . .	Not under 37°	203 to 212
White floating soap .	Not under 35°	Not under 212
Special laundry soap .	Not under 35°	——
Ordinary laundry soap	Not under 33°	——
Salt water soap .	——	Not under 256

Generally speaking, the higher the titer test, the harder the soap. The table on p. 196 gives some constants of the fatty acids for comparison.

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[1] *J.S.C.I.*, 1918, 37, 262 T.

(*n*) *Origin of the Fatty Acids.*—It will not often be necessary to determine the nature of the fat from which the soap is derived. As mentioned above, if an appreciable quantity of soluble fatty acids is found by the process described under (*e*), the soap probably contains fatty acids derived from coconut or palm kernel oils. In some cases it may be possible to determine the origin of the fatty acids by determining such constants as the solidifying point (titer test), saponification value, iodine value and specific gravity, as described in Chapter III.

The Results of the Analysis.—The accompanying table contains typical analyses of various grades of soap, most of which are by C. Hope. As a rule, the most important of the determinations described above are those of water, alkali combined as soap, to be calculated as Na_2O, free caustic alkali as NaOH (or KOH), sodium (or potassium) carbonate, water insoluble material, *i.e.*, fillers or adulterants (see under (*k*)), fatty acids, calculated as anhydrides, and rosin acids.

THE DETERMINATION OF PHENOLS AND NEUTRAL HYDROCARBONS IN CARBOLIC SOAPS.

The following method for the analysis of carbolic soaps is due to Allen: Five grams of the sample are dissolved in warm water, and 30 c.c. of a 10 per cent. solution of sodium hydroxide are added. The alkaline solution is transferred to a separating funnel and extracted with ether. The ethereal solution is evaporated in a tared flask on the water bath, and the residue weighed; this residue, which will consist of neutral oils of tar or other substances of a like nature which may have been added to the soap, may be appreciably volatile at 100°, in which case it will be difficult to get an accurate estimate of its amount.

Regarding the ether extraction, it may be pointed out that unless a large excess of caustic soda is used, some of the phenols may be extracted from the solution of their sodium salts owing to their weakly acidic nature and the consequent liability of their salts to be hydrolysed (cf. p. 90). Troublesome emulsions during the extraction may be avoided to some extent by not shaking too violently, and, if necessary, alcohol in amounts up to one-fifth of the total volume may be added to break them down. If it is desired to get an accurate estimate of the neutral oils, the united ether extracts should be washed with water to remove the soap dissolved by the ether, and the residue should be dried by heating the containing flask to 50° while passing a current of dry air through it.

The alkaline liquid which has been separated from the ether is transferred to a more capacious separating funnel and treated with an excess of saturated brine, which will precipitate the soaps while the phenols remain in solution. After agitating to coagulate the soap, the liquid is filtered; in case the soap should not coagulate easily, the addition of a little tallow or palm oil soap dissolved in water will often facilitate the separation. The precipitated soap is washed twice with strong brine, and the washings are added to the main filtrate, which is then diluted to 1 litre. 100 c.c. of this solution, which will be equivalent to 0·5 gram of soap, are placed in a 500 c.c. stoppered bottle and acidulated with sulphuric acid, when it should remain perfectly clear; if precipitation occurs at this stage, the fatty acids will not have been completely removed. In such cases 200 c.c. of the

Description.	Fatty and Rosin Anhydrides.	Na_2O as Soap.	Si_2O.	Na_2O as Silicate.	Na_2CO_3 and NaOH.	NaCl.	Na_2SO_4.	CaO and Fe_2O_3	H_2O	Total.	Fatty and Rosin Acids.	Origin.
White I. . .	69·06	8·96	0·01	*nil*	0·27	0·49	0·16	0·07	21·14	100·18	71·20	Tallow.
White IV. . .	44·27	6·23	7·02	2·36	0·75	0·32	0·34	0·34	38·14	99·77	45·64	Tallow and coconut oil.
Cold water I. .	71·30	7·98	1·07	0·48	0·75	0·36	0·30	0·16	17·44	99·84	73·50	Tallow rosin and cotton oil.
Cold water II..	49·95	7·00	2·34	1·01	0·33	0·51	*nil*	0·50	38·18	99·82	51·50	Tallow rosin and cotton oil.
Marseilles I. .	62·66	7·27	0·06	0·03	0·77	0·76	0·30	0·16	28·20	100·21	64·60	Chiefly olive oil.
Palm oil I.. .	59·28	6·65	0·42	0·01	0·39	0·47	0·13	0·16	32·35	99·86	61·08	Palm oil.
Mottled . .	38·89	5·76	6·40	1·29	1·62	1·78	0·72	0·03	38·70	95·19	40·10	Palm kernel oil.
Pale rosin I. .	60·90	7·22	0·04	*nil*	0·10	0·46	0·12	0·02	31·22	100·08	62·78	Tallow and rosin.
Pale rosin III.	39·92	4·70	0·62	0·25	0·20	1·48	0·18	0·15	52·40	99·90	41·15	ditto.
"Yellow" for foreign markets	10·90	1·36	0·03	*nil*	trace	2·57	0·56	0·14	84·00	99·56	11·20	ditto.
Marine for emigrants .	19·42	3·11	9·00	3·98	3·00	5·13	0·35	0·16	53·52	99·47	20·02	Palm kernel oil.
White Castille	76·7	9·14	—	—	—	0·36	—	0·09	13·25	100·54	—	—

alkaline liquid should be taken, and powdered salt dissolved in it to saturation; the solution thus obtained should then be filtered through a dry filter, and 100 c.c. of the filtrate acidified as before. The phenol is then determined by titration with standard bromine water (see below), which is run in from a burette, the stopper of the bottle being replaced and the contents agitated after each addition. The end point is reached when the solution acquires a permanent faint yellow tint. If crystallised phenol has been introduced into the soap the precipitated tribromophenol will be precipitated in snow-white flocks which allow the faintest tint owing to excess of bromine to be observed quite readily. If, on the other hand, cresylic acid (*i.e.*, the cresols) has been added, the precipitate will be milky and will not separate well from the liquid, though the end point can still be observed. The addition to the original solution of a known amount of pure phenol may cause the precipitate to coagulate, in which case the yellow colour due to excess of bromine will be more easily seen. This addition must, of course, be allowed for in calculating the result of the analysis.

The bromine solution which is used for the titration is conveniently prepared by mixing in a stoppered bottle one measure of saturated bromine water with two measures of water. The resulting solution, which is approximately of 1 per cent. strength, should be standardised immediately after use by means of a standard solution of phenol of the quality indicated by the analysis to be present in the sample. The phenol solution may be made by dissolving 0·5 gram of the phenol (usually either Calvert's No. 2 or No. 5 carbolic acid) in 20 c.c. of a 10 per cent. solution of sodium hydroxide with

5 grams of a non-phenolic soap. The solution is precipitated with brine in the same way as the solution of the sample under examination, the filtrate diluted to 1 litre, and 100 c.c. acidulated with sulphuric acid and titrated with the bromine water.

Carbolic soaps are often sold as containing 20 or 30 per cent. of carbolic acid; they may contain anything from 1 to 30 per cent. of phenol, derived either from pure crystallised phenol or the common cresylic acids. According to Allen, the proportion of phenol found is occasionally less than that stated to be present, the difference probably being due to loss by evaporation.

PHENOLIC DISINFECTANTS CONTAINING SOAP.

The preparations to be dealt with under this heading consist of creosote oil or coal tar phenols, chiefly cresols (cresylic acid), to which soap has been added in order to form an emulsion or a homogeneous product which shall be readily miscible with water. As examples we may take the preparations known as creoline and lysol. The former consists of creosote oil and an aqueous solution of soda rosin soap, which have been mixed to form an emulsion. Being made from the crude creosote oil, it may contain some 40 to 60 per cent. of neutral hydrocarbons and about 10 to 20 per cent. of cresylic acid; the better the quality, the less of the former and the more of the latter will it contain. Creoline should form a permanent emulsion when mixed with water in the proportion of 1 to 40, the greater part of the cresylic acid dissolving as such, or as sodium salts, and the neutral hydrocarbons remaining suspended in the form of minute globules owing to the emulsifying action of the soap.

Lysol and sapocarbol, on the other hand, are prepared by mixing cresylic acid containing relatively small amounts of hydrocarbons, with rosin or a fatty oil such as linseed oil, and saponifying the rosin or oil by treating the mixture with a solution of potash in water and alcohol. The viscous brown transparent liquid thus obtained is readily soluble in water. Lysol should contain at least 40 to 50 per cent. of phenols, and at most only about 3 or 4 per cent. of hydrocarbons, in order that it may dissolve in water as completely as possible.

Besides the products just described, there are a number of other similar preparations on the market containing varying proportions of cresylic acid, soap and hydrocarbons. Among these may be mentioned sanatol, saprol, solutol and salveol. Many sheep dips are similar in composition to the above, and may be analysed by the methods given below. The latter preparations sometimes contain sodium arsenate or sodium carbonate in addition to or in place of the soap.

Concerning the antiseptic values of the various constituents of the coal tar oils, see p. 92.

The Analysis of Creoline, Lysol, Sheep Dips, etc.—The estimation of neutral oils of tar, coal tar phenols, pyridine bases and fatty or rosin acids may be carried out as follows: 200 grams of the sample are made distinctly acid by the addition of sulphuric acid (1 to 2), and agitated with ether in a separating funnel; after separating, the aqueous layer is again extracted with two successive portions of ether. If sufficient coal tar oil is present to dissolve the phenols and fatty or rosin acids, the addition of ether may be omitted. The pyridine bases may be estimated in the acid liquid, as described in Chapter II., under the "Testing of Creosoting Liquor."

The phenol and cresols may conveniently be separated from the fatty or rosin acids by the method recommended by Fox and Barker.[1] The mixture is distilled in a Würtz flask up to 220°, when all the phenol and most of the cresols distil over together with some or all of the tar oils, the fatty acids being left behind practically unchanged. The phenols and neutral oils which have distilled may then be estimated by the methods described for creosoting liquor on p. 95 *et seq.*

Soda or potash may be estimated by burning off about 5 grams of the original sample in a platinum crucible or dish, treating the residue with successive small portions of concentrated sulphuric acid, and weighing the ignited sodium or potassium sulphate in the usual manner.

REFERENCES.

"The Chemical Technology and Analysis of Oils, Fats and Waxes," by J. Lewkowitsch, 3 vols. 1913-1915. Macmillan & Co., London.

Allen's "Commercial Organic Analysis," Vol. II. 1910. J. and A. Churchill, London.

"Engineering Chemistry," by Stillmann. 1910. Williams and Norgate, London, and the Chemical Publishing Co., Easton, Pa.

"Chemisch-Technische Analyse," Vol. I., by Post. 1908. Fr. Vieweg, Brunswick.

"Einheitsmethoden zur Untersuchung von Fetten, Oelen, Seifen und Glyzerinen, sowie sonstigen Materialen der Seifenindustrie," by Donath and Margosches (Sammlung Chemischer und Chemisch-Technische Vorträge).

"Technologie der Fette und Oele," by Gustav Hefter. 3 vols. 1906 to 1910. J. Springer, Berlin.

"The Soap Maker's Handbook," edited chiefly from the German of Deite, Engelhardt and Wiltner, by W. Braunt. 1912.

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[1] *J.S.C.I.*, 1918, 37, 266 T.

E. F. Spon, Ltd., London; Henry Carey, Baird & Co., Philadelphia.

"Traité de Savonnerie," by E. Moride. 1909. Librairie Polytechnique, Paris.

"Textile Soaps and Oils," by Hurst. 1914. Scott Greenwood, London.

"Soaps," by Hurst. 1907. Scott Greenwood, London.

"Modern Soaps, Candles and Glycerine," by Lamborn. 1916.

"Handbook of Soap Manufacture," by Simmon and Appleton. 1908.

"A Practical Handbook on the Manufacture of Hard and Soft Soaps," by Watt. 1907.

"Technical Methods of Chemical Analysis," by Lunge, trans. by Keane. 1911. Gurney and Jackson, London.

Also other works mentioned at the end of Chapter III.

CHAPTER V

PETROLEUM AND ITS DISTILLATION PRODUCTS—LUBRICATING OILS

INTRODUCTORY

THE petroleums, or mineral oils, are usually more or less viscous, dark brown or black fluids having specific gravities lying between 0·73 and 0·97. Chemically, they consist of complex mixtures of hydrocarbons together with smaller quantities of oxygen, nitrogen and sulphur compounds; the nature of the hydrocarbons varies with the locality from which the petroleum is obtained, and, to a smaller extent, with the age of the well. Most petroleums distil over a wide range of temperature and yield a variety of important products such as motor spirit, burning oil, lubricating oil, etc., while the residues which remain after distilling off the more volatile portions have in recent years found a most important use as fuel for internal combustion engines.

Composition of the Petroleums.—As examples, we may take the petroleums of Pennsylvania, U.S.A., and the Caucasus, which together constitute by far the greater part of the world's supply of this commodity.

Pennsylvania petroleum, which usually has a specific gravity lying between 0·80 and 0·87, consists chiefly of paraffins from pentane C_5H_{12} (b.p. 38°) up to the solid $C_{20}H_{42}$; higher members of the series, such as $C_{25}H_{52}$

and $C_{30}H_{62}$, are also known to be present. The portions boiling above 200° probably contain olefines in addition to the paraffins; as is also the case with the other petroleums, many of the higher boiling constituents are of unknown constitution. Aromatic hydrocarbons are present only in small amount. Pennsylvania petroleum is accompanied by hydrogen and the members of the paraffin series which are gaseous at the ordinary temperature.

Caucasian petroleum has a higher specific gravity than Pennsylvania petroleum, usually from 0·88 to 0·94, and differs widely from the latter in chemical composition. According to Markownikoff, it contains at least 80 per cent. of naphthenes, *i.e.*, polymethylenes and their alkyl derivatives, among which pentamethylene, hexamethylene, methyl hexamethylene and other similar compounds of known constitution have been recognised. The naphthenes resemble the paraffins in being saturated bodies which are not acted on by concentrated sulphuric acid or potassium permanganate; with the halogens and dilute nitric acid, they yield halogen and nitro substitution products respectively. In addition to the naphthenes, Russian petroleum contains about 10 per cent. of aromatic hydrocarbons, and less than 1 per cent. of paraffins. The higher boiling fractions appear to contain unsaturated substances such as olefines and naphthylenes, the latter being olefine analogues of the naphthenes. Like Pennsylvania oil, Russian oil contains a number of substances of unknown constitution, especially in the higher boiling fractions.

From the foregoing it will be gathered that the two classes of petroleums described show important differences in their behaviour on distillation. Generally speaking, Pennsylvania petroleum distils over a wider range of temperature, and contains a much larger proportion of volatile matter than Russian petroleum, the residue from the latter being larger in amount and more fluid in consistency than that from the former. The primary products of distillation of the petroleums are as follows: (1) Up to 150°, light oils or mineral naphtha, specific gravity 0·65 to 0·67, from which are obtained by fractional distillation various mixtures of light hydrocarbons which are used as fuel for motor engines, extraction and cleaning purposes, and burning naphtha. (2) 150° to 300°, illuminating oils, such as kerosene or solar oil, specific gravity 0·75 to 0·87. (3) Residuum, which in the case of Russian oils is generally used as fuel, and in the case of Pennsylvania oil, distilled under vacuum or ordinary pressure for lubricating oils, the residue in the latter case being worked up for vaseline, solid paraffins or paving asphalt. If the distillation is carried to a finish, the residue consists of a coke-like material which may be used as fuel.

The method of distillation depends on the nature of the petroleum and the nature of the products required. Russian petroleum is distilled by the continuous process, the material passing through a series of stills kept at progressively increasing temperatures; the distillate is divided into two portions, gasolene and kerosene. The former is redistilled till the distillate has a specific gravity of 0·750, and the residue added to the main kerosene fraction, which is distilled till the specific gravity of the distillate reaches about 0·825. The total residue, which has a specific gravity of 0·903, if distilled, will yield about 10 per cent. of solar oil, of specific gravity 0·86 and flash point 105° F., as determined by the Pensky-Martens apparatus, and about 35 per cent. of lubricating oils, the final residue being used as fuel. Compared with Pennsylvania petroleum, Russian petroleum yields a relatively small proportion of light petroleum and burning oil, the yield of the former, which includes benzine of specific gravity about 0·725, and heavy benzine or gasolene of specific gravity about 0·770, being about 4 to 6 per cent., and of burning oils, about 27 per cent. of kerosene of specific gravity about 0·822 and 5 per cent. of solar oil of specific gravity about 0·86 to 0·88. At Baku, about 30 to 40 per cent. is generally distilled off, the residue, known as "astatki," being used as liquid fuel.

Pennsylvania petroleum cannot be distilled by the continuous process, owing to the large amount of burning oil which it yields, and the viscous nature of the residue. When submitted to the simple distillation process, it first yields gaseous paraffins which are either burnt as fuel or condensed by pressure and cooled by expansion ; owing to the growing scarcity of the lighter petroleum fractions, the latter procedure has become more common in recent years ; the gasoline is formed by the condensation of the heavier hydrocarbons of the gas, while some of the lighter gaseous constituents dissolve in the liquid. Cymogene, b.p. 0°, and rhigolene, b.p. 18°, are used in surgery for freezing. The light petroleum or petroleum naphtha fraction is taken till the specific gravity of the distillate reaches 0·725 to 0·750 ; it includes gasoline, specific gravity 0·625, petroleum ether, specific gravity 0·665, naphtha, specific gravity 0·676, and benzine,[1] specific gravity 0·736, these amounting to 9 to 18 per cent. of the total. The kerosene fraction is taken until the specific gravity of the distillate reaches 0·840 to 0·845, the average yield of burning oils being about 55 per cent. The residue is transferred to other stills and distilled for lubricating oils, the average yield of the latter, if distilled under ordinary pressure, being about 17 per cent. The solid paraffin amounts to about 2 per cent., and coke residue about 10 per cent. Distillation of the residue by the vacuum process gives a much larger yield of lubricating oils and vaseline.

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[1] Distinction must be made between benzine, which is obtained from petroleum or mineral naphtha, and benzene, the aromatic hydrocarbon obtained from benzol, the distillation product of coal tar naphtha. The terms petroleum spirit and light petroleum are also used to designate the more volatile distillation products of petroleum naphtha.

Lighter oils may be produced from heavier oils by a process of destructive distillation known as "cracking." The vapours of the oil are allowed to condense on the walls of the still, and on falling back into the hot residue they are decomposed. The nature of the products depends on the temperature, pressure, and the relative volumes of liquid and gas in the retort. At the highest temperatures gas and coke are obtained. In order to obtain a maximum yield of petrol-like products which are nowadays in great demand, it is necessary to use low temperatures and high pressures. Certain substances, such as colloidal graphite, appear to have a catalytic action furthering decomposition, and their use admits of lower temperatures being employed. If the volume of the liquid is about one-third to one-half of that of the gas or vapour in the retort, and the pressure 50 to 60 kg. per sq. cm., a 50 to 70 per cent. yield is obtained of a product which is usable as petrol and has a specific gravity of 0·700 to 0·710, together with gas and coke. Benzene and toluene are among the products obtainable; the experience of recent years has shown that benzol forms an efficient substitute for petrol. This is a point of importance, as the demand for petrol has increased enormously, especially since the development of aircraft. (See also p. 39.)

Regarding the petroleums from other parts of the world, the Galician and Roumanian oils are intermediate between the Pennsylvania oils on the one hand, and the Russian oils on the other, in respect to chemical composition and general properties. The specific gravity of these oils is usually about 0·870. The petroleums obtained from Germany and Ohio, U.S.A., also contain both paraffins and naphthenes. Californian petroleum contains only a small percentage of low boiling constituents; it contains no paraffins, but naphthenes and aromatic hydrocarbons, the latter often being present in considerable amount. Appreciable quantities of oxygen and nitrogen compounds are also present. Texan petroleum does not, as a rule, commence to distil below 240°, and consists chiefly of unsaturated hydrocarbons of high molecular weight. Canadian petroleum contains paraffins and olefines, more aromatic hydrocarbons and less light paraffins than Pennsylvania oil. Indian petroleum consists chiefly of naphthenes, with some benzenoid hydrocarbons.

In 1914 the United States produced about two-thirds of the world's supply of petroleum, Russia about 16 per cent., Mexico 6 per cent., Roumania 3 per cent., and other countries percentages varying from one-half to two and a half. The greater part of the world's supply of burning oil and light petroleum, or benzine, is obtained from the American, and some of the Roumanian and Galician oils. The lubricating oils obtained from these are generally heavier, more viscous and of higher solidifying point than those obtained from the Russian oils

Oils for lubricating steam cylinders, which require to be fairly thick and viscous on account of the relatively high temperatures at which they are used, are therefore obtained best from the American petroleums. Crude petroleums containing little or no volatile hydrocarbons coming under the heading of light petroleum or burning oils, will, no doubt, find increasing use as liquid fuel for internal combustion engines of the Diesel type, the only preparation necessary being the removal of water and solid matter by the settling out process to which crude oils are generally subjected. The use of Russian petroleum residue as liquid fuel has already been alluded to.

The various fractions obtained by the distillation of crude petroleum are refined, where necessary, by treatment with limited quantities of concentrated sulphuric acid, and subsequent washing with dilute caustic soda solution and water. Impurities which colour the oil and give it an objectionable odour are thus removed. If sulphur compounds are present in appreciable quantity, they are removed generally by treatment with lead or copper oxide; either the fractions are redistilled over the metallic oxide, or the vapours are allowed to pass over it. In Canada, burning oils are desulphurised by treatment with a solution of litharge in caustic soda solution.

THE EXAMINATION OF CRUDE PETROLEUM.

Owing to the complex nature of crude petroleums, these are but rarely examined by purely chemical methods in technical laboratories. By means of the distillation test, described below, the nature and amount of the distillate to be expected may be determined, and the oil may be roughly classified. The specific gravity may also be used to help in classifying and comparing petroleums. The determination of extraneous matter, *i.e.*, water and sediment, will also be described.

Water.—A weighed quantity of the oil, about 100 to 200 grams, is mixed with about a third of its bulk of toluene, and distilled from a flask heated in an oil bath; the distillate is collected in a graduated cylinder. The distillation is stopped when no more water is seen to come over, and the vessel containing the distillate is warmed and gently shaken so that the water adhering to the sides falls to the bottom. The volume of the layer of water at the bottom of the cylinder is read off after cooling.

The water may also be distilled off for measurement without the addition of toluene. Shewsbury[1] recommends the following method: A 500 c.c. distilling flask is fixed so that the side tube is at right angles to the bench and inserted into a 25 c.c. cylinder graduated in tenths or fifths, the bore of which is not much greater than the diameter of the side tube. The cylinder stands almost completely immersed in a vessel which is cooled by running water. 100 c.c. of the oil, or a volume equivalent to 100 grams, is placed in the flask with a few pieces of dry pumice. The surface of the flask uncovered by the oil is first heated by a Bunsen flame which is kept in constant motion, the heating being continued downwards from above, occasionally heating below the surface of the oil. The process is finished by distilling a few c.c. of the oil itself, taking care not to allow any condensed drops to roll back into the hot oil, and to keep the bottom of the side tube above the level of the distillate. The cylinder is finally rotated in order to assist the settling out of the water, and petroleum ether may be added to accelerate this process. The percentage of water is read off when no further increase in volume occurs.

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[1] *Analyst*, 1914, 529; see also Allen and Jacobs, Technical Paper, 25, U.S.A. Dept. of Interior, Bureau of Mines, 1912.

Other methods have been based on the measurement of the volume of gas evolved by the action of calcium carbide or sodium. Calcium carbide gives low results owing to the solubility of acetylene in the oil.

Sediment.—5 to 10 grams of the oil are well mixed with 100 to 500 grams of benzene and allowed to stand overnight ; the benzene and petroleum mixture is then filtered through a weighed filter, and the insoluble residue washed with benzene, dried at 100° and weighed. The sediment estimated will consist of mineral matter, and will not include any solid pitch or asphalt which may exist in the oil, these being soluble in benzene.

Specific Gravity.—If the oil is not too thick, a hydrometer may be used. The oil is poured into a cylinder of suitable size and allowed to stand for some time to acquire the room temperature. The float is allowed to sink gradually into the oil, and its level read after fifteen minutes, the reading corresponding with the level of the liquid being taken. In the case of dark oils, the level to which the oil rises up the stem of the float is read off, and 0·0015 or 0·0010 is added to the reading, according as the paper scale is shorter or longer than 16 cm. The temperature may be read off from the thermometer on the float, or, failing this, the room temperature may be taken. In order to reduce the reading to 15°, a correction of 0·00065 per degree Centigrade may be applied.

If the specific gravity bottle is used, and the oil is thick, the bottle should first be nearly filled with the oil and allowed to stand in a warm place overnight, in order that all air bubbles may rise to the top. The bottle and its contents are then cooled to 15° by allowing to stand in a water bath at that temperature, filled up and weighed in the usual way.

Distillation Test.—The test to be described is originally due to Engler, certain modifications having been introduced by Ubbelohde. The method is empirical, but gives uniform results if the prescribed dimensions of the apparatus and conditions of distillation are adhered to. If the oil contains much water, it should first be dehydrated by means of calcium chloride, to avoid frothing in the distillation. 100 c.c. of the oil are distilled from a flask of the form and dimensions shown in Fig. 15. A Liebig condenser may be used, and the fractions are collected so that they may be weighed or measured. The distillation should be carried out so that the distillate collects at a uniform rate of two drops per second. At first the flask should be heated on wire gauze, and subsequently a free flame may be used. The first, or naphtha fraction, is collected until the temperature indicated by the thermometer is 150°; the receiver is then changed for the kerosene fraction, which is collected between 150° and 300°. In the case of Russian and Galician oils, the kerosene fraction should only be collected up to 285° and 275° respectively, the oils obtained at higher temperatures from these petroleums being unsuitable for burning in lamps as they cause excessive charring of the wick. What remains in the flask after distilling off the kerosene is weighed as residuum. If desired, separate fractions may be taken at more frequent intervals, say, every 25° ; this is, however, only advisable if the yield of distillate is large. If the oil only yields very little distillate up to 300°, a larger flask,

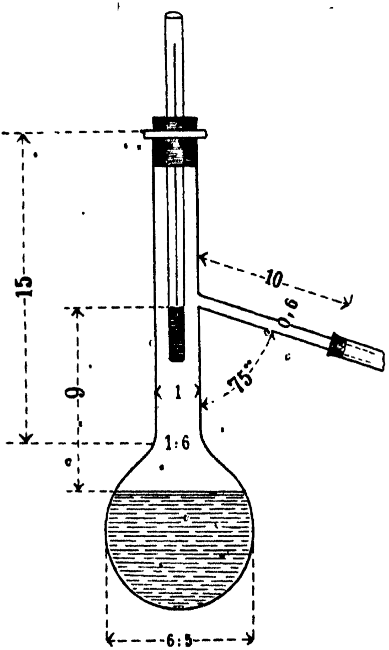

FIG. 15.—Petroleum Distillation Flask according to Engler. Dimensions in cm.

say of 140 c.c. capacity, must be used, or the quantity of oil taken must be reduced to 80 or 90 c.c. ; the heated oil will otherwise expand so as to fill the flask to such an extent that it may be carried over mechanically during the distillation. The same precautions must be taken if the material under examination consists of petroleum residue.

Various petroleums of known origin should be compared by this method. Petroleum is examined for excise purposes in specially constructed metal stills of standard dimensions. For a method of examining petroleum for technical purposes, duplicating the manufacturing operations on a small scale, see Holde's work mentioned at the end of this chapter.

The following table gives the specific gravities and distillation results of some petroleums :—

	Specific gravity.	Petrol fraction per cent.	Kerosene fraction including up to 350°, per cent.	Lubricating oil fractions and Residues, per cent.
Pennsylvania	0·79—0·82	10—20	55—70	10—20
Galicia . .	0·82—0·90	5—20	35—50	30—45
Ohio . .	0·80—0·90	10—20	30—40	35—50
Baku . .	0·83—0·90	2—10	25—35	50—65

THE EXAMINATION OF PETROLEUM NAPHTHA AND ITS DISTILLATION PRODUCTS.

Petroleum naphtha is usually understood to include the portion of the distillate from crude petroleum which comes over below 150°; on redistillation, however, it is generally found to contain up to 10 per cent. of oils distilling above this temperature. Motor spirit was formerly understood to be derived from the fraction distilled up to about 150°, but of later years portions from the higher boiling kerosene fraction have been included in it owing to the demand for the lighter spirit exceeding the supply. Petrol products consist chiefly of either naphthenes or paraffins, or mixtures of these substances in any proportion. Products obtained by crack distillation are characterised by the presence of notable amounts of unsaturated hydrocarbons.

The various products which are obtained by the fractional distillation of petroleum naphtha are best characterised by their *specific gravity*, which may be determined by means of a float or the Westphal balance, which is described in Chapter II., p. 50. The results may be calculated to 15° C. from the data given in the following table, which is due to Mendeléeff :—

For Specific Gravities from	Correction per ° C.
0·700 to 0·720	0·000820
0·720 ,, 0·740	0·000818
0·740 ,, 0·760	0·000800
0·760 ,, 0·780	0·000790
0·780 ,, 0·800	0·000780

The most important uses of the petroleum naphtha distillates are as fuel in internal combustion engines, and as solvents for extraction of organic material, as, for example, the extraction of fatty oils and fats from seeds and other vegetable products.

Formerly, petroleum spirit of specific gravity about 0·680 was used for internal combustion engines, but with the rapid development of the motor industry heavier grades came to be used, and this tendency was accentuated during the war. The specific gravities of Nos. 1, 2, and 3 war spirits are about 0·725, 0·730, and 0·740 respectively. "Bus spirit," which is useful only for heavier vehicles, has a specific gravity of 0·765 or even higher. The specific gravity is no absolute criterion taken by itself, for a normal average specific gravity would be shown by a mixture of light and heavy petrol fractions, while the presence of benzol, either natural or added, would materially raise the specific gravity (see p. 55), and a mixture of this description would be decidedly superior to a purely aliphatic petrol fraction of corresponding specific gravity. The presence of naphthenes also raises the specific gravity, thus cyclohexane has a specific gravity of 0·783.

Colour and Foreign Matter.—Petroleum spirit or petrol should be water white and free from objectionable odour. Objectionable odour is sometimes masked by the addition of small quantities of turpentine or rosin oil and treatment with alkali. *Water* in small amounts is easily seen in the sample. *Acids or alkalies or hydrogen sulphide* may easily be detected in an aqueous extract; if sulphuric acid should be found it will probably be acid which has not been removed after the refining process. *Sulphur.*—Blount[1] states that of the methods in use for the determination of sulphur in petrol, the Carius method is the only dependable one, and will give good results with close and careful work. It must be remembered that very high pressure will be developed in the sealed tube. According to Blount, the sulphur in petrol usually amounts to less than 0·1 per cent.

The above directions may also be used in the examination of motor benzol (see p. 71). A high sulphur content will give rise to corrosion of the internal parts of the engine owing to the sulphurous acid which is formed.

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[1] *Analyst*, 1918, 43, 89.

Fractional Distillation Test.—Certain rough preliminary tests have been proposed: for example, a good petrol should leave no oily mark when evaporated on filter paper, or the time taken for the spontaneous evaporation of 10 c.c. in a porcelain dish may be noted, the times varying from under two hours to over four hours. For accurate information, however, the petrol should be fractionally distilled. The following method, recommended by Anfilogoff,[1] resembles those devised by Ubbelohde and Holde and by Redwood. 100 c.c. of the sample are distilled from a 150 c.c. Engler flask (see p. 212) connected with a condenser having a 24-inch straight glass inner tube and an 18-inch jacket; the inlet end of the inner tube should preferably not be enlarged. The thermometer, which is preferably not calibrated for the first four inches, is not placed in the usual position shown in Fig. 15, but with the top of the bulb half an inch below the outlet of the side tube; the readings are thus rendered more independent of the effect of slight draughts. The method further differs from that of Ubbelohde and Holde in that the initial boiling point is noted at the temperature at which the first drop falls from the thermometer and not from the outlet end of the condenser. Distillation is carried out at the rate of two drops per second till the bottom of the flask is dry, the process occupying twenty minutes. The results are claimed to be not very divergent from those obtainable with the use of a Young eight pear column (see p. 52) and to represent the same percentages of distillates as are obtainable on a large scale. The volume of distillate obtained is usually measured every ten degrees, the percentage volume obtained up to 100° being the most important. Kissling[1] recommends for the testing of petrol for small quantities of high boiling constituents that the distillation should be stopped when 95 per cent. has distilled, and the residue be transferred to a clock glass and allowed to evaporate for five to ten hours at the ordinary temperature. A fairly thick oil is obtained from inferior grades of petrol by this method.

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[1] *J.S.C.I.*, 1918, 37, 21 T.

If an unduly large proportion of high boiling constituents are present, the petrol will not evaporate with sufficient rapidity on entering the explosion chamber, especially in cold weather. It is also obvious that petrol spirit which is to be used for extraction purposes must not contain high boiling constituents or it will only be removed with difficulty from the extracted material. Formerly it was considered that a good motor petrol should be completely volatile below 100° or only yield about 5 per cent. above this temperature. Requirements are less stringent nowadays; for example, Anfilogoff found by his method 41, 31, and 21 per cent. volatile up to 100° in the cases of Nos. 1, 2, and 3 war spirits respectively, and 6 and 10 per cent. for bus spirits. Under present conditions motor spirit completely volatile from 170° to 180° would be considered good.

Benzol in Petrol.—Aromatic hydrocarbons are present as natural constituents in many petroleums (see pp. 203 to 207), and mixtures containing added benzol are likely to be met with in future with increasing frequency owing to the recognition of benzol as an efficient motor fuel.

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[1] Chem. Zeit., 1908, abs. *Analyst*, 1908, 368.

Aromatic hydrocarbons may be detected by their smell, and their presence may also be indicated by the sample having a relatively high specific gravity (see pp. 215 and 55) and yet giving far better results in the distillation test than would be expected from a petrol consisting only of aliphatic hydrocarbons of the same specific gravity. The effect of naphthenes which are present in varying amounts in many petrols is similar (see p. 215, and below).

According to Holde, aromatic hydrocarbons may be detected as follows: A small quantity of the petrol is treated with a little powdered asphalt (sufficient to cover the point of a penknife), which has been freed from mineral matter (by solution in benzene and filtration) and repeatedly washed with petroleum ether of specific gravity 0·70 to 0·71 in order to free it from its more soluble constituents. The petrol containing the asphalt is passed through a small filter into a test tube; if the filtrate is colourless, benzene hydrocarbons are absent; if brown, benzene or toluene are probably present.

If a few cubic centimetres of light petroleum containing benzene or toluene be shaken in a test tube with a mixture of concentrated nitric and sulphuric acids, the mixture will sooner or later become warm, while the upper layer will develop a yellow coloration; on pouring into water, there will be a separation of yellow, oily drops, having the characteristic odour of nitro-benzene.

Formanek has proposed the use of indanthrene dyes which are soluble in aromatic hydrocarbons but not in aliphatic hydrocarbons, on the same lines as the asphalt test just described; a series of standards of known composition are made for purposes of comparison.

Dragon's blood may be used in a similar way, shaking up 0·2 gm. of dragon's blood with 30 c.c. of the sample in a small stoppered bottle; petrol or kerosene only become coloured faintly pink while the aromatic hydrocarbons take up a deep red colour. These colorimetric tests only give very rough indications.

The estimation of benzene and toluene in petroleum or petrol by the sulphonation method (cf. pp. 65 and 66) has been carefully studied by Thole,[1] who recommends the use of 98 per cent. sulphuric acid for the reason that fuming acid attacks the naphthenes and some of the paraffins to a certain extent. As the result of a number of experiments, the temperature limits of 40° to 95° were fixed on for collecting the fraction in which the benzene is to be estimated, and 95° to 122° for the toluene fraction. These "cutting points" were so chosen that the benzene which is missing from the benzene fraction shall be as nearly as possible balanced by the amount of toluene which passes over too soon, and similarly the amount of toluene which is missing in the toluene fraction is balanced by the benzene and xylene which find their way into this fraction. This scheme is claimed to be satisfactory for mixtures containing up to 30 per cent. of aromatic hydrocarbons. The mixtures were distilled through a five-section Young Thomas fractionating column at the rate of one drop per second, which is lowered to two drops in three seconds when a temperature within three degrees of the cutting point was reached.

The procedure of directly measuring the unattacked hydrocarbons (see pp. 65 and 66) was not adopted owing to the experimental error to which such processes are subject, one disturbing factor being the appreciable solubility of non-aromatic hydrocarbons in the acid solution of sulphonic acids. The method is analogous to the one described on p. 63 for the determination of carbon disulphide in benzol. Three volumes of 98 per cent. sulphuric acid and one volume of the sample are shaken vigorously together in a stoppered 50 c.c. cylinder at frequent intervals during half an hour. The mixture need not be cooled unless the sample was rich in aromatic hydrocarbons. *i.e.*, containing more than 50 per cent., in which case it may also be necessary to separate the supernatant spirit and treat it with a fresh quantity of acid. The percentages of benzene or toluene are calculated from the specific gravities of the spirit before and after treatment with sulphuric acid, taken by means of a Sprengel pyknometer :—

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[1] *J.S.C.I.*, 1919, 38, 39 T.

$$\text{Aromatic} = \frac{\text{Initial sp. gr.} - \text{Final sp. gr.}}{\text{Sp. gr. of aromatic} - \text{Final sp. gr.}}$$

The specific gravities used were: benzene, 15·5°/4°, 0·8841, toluene, 0·8712. It is not necessary to wait for complete separation of the layers, as all that is required is a sample of the supernatant spirit suitable for a specific gravity determination. It is shown that the error which might possibly arise through the naphthenes being more readily soluble in the sulphonic acid solution than the paraffins is comparatively small. Regarding *the composition of the non-aromatic portion*, the specific gravities of the 40° to 95° fractions after treatment with sulphuric acid varied from 0·688 to 0·722, and the 95° to 122° fractions from 0·718 to 0·747, the lower specific gravities indicating the presence of paraffins and the higher ones the predominance of naphthenes.

Olefines. It must be remembered that unsaturated hydrocarbons are absorbed by strong or fuming sulphuric acid, and complications may thus be introduced into the above and similar methods. The amounts of olefines contained in straight distilled petroleum spirits are however generally negligible, but in spirits obtained by crack distillation they may be considerable. This point is illustrated by the iodine values (see p. 126) of the different products. Thus the iodine values of straight distilled petroleum naphthas have been found to range between 0 and 7, and those of cracked products between 75 and 100 in the case of the light spirit distillates, and between 65 and 75 in the case of the illuminating oil distillates.[1] Small amounts of olefines may easily be detected by their action on potassium permanganate.

Turpentine and Rosin Oils.—Holde recommends the following test for small quantities of turpentine or rosin oils, either in pure petrol or in petrol containing benzene or homologues of the latter. Bromine vapour is allowed to flow into a test tube containing a little of the benzine; on shaking the benzine should immediately take up the red colour of the bromine; if, however, turpentine or rosin oils be present, traces of added bromine will rapidly be absorbed, owing to the unsaturated nature of these substances. This test may, however, be masked by the presence of the olefines contained in cracked products.

THE EXAMINATION OF KEROSENE OR BURNING OIL.

Kerosene, or oil for burning in wick lamps, is obtained by redistilling the fraction which succeeds the naphtha fraction in the distillation of crude petroleum. The

[1] Comptes Rendus, 1910, 150, 1338.

refining process usually consists in treatment with strong sulphuric acid and subsequent washing with dilute alkali and water, and, where necessary, desulphurisation as indicated above. The specific gravity of American kerosene usually lies between 0·790 and 0·800, and that of Russian kerosene between 0·821 and 0·823, the latter generally being a more homogeneous product than the former. A parallel product obtained from shale oil has a specific gravity of about 0·800. A good kerosene should be water white, or, at the most, of a light yellow colour. Although the degree of colour of burning oil is often made the basis of commercial transactions, this cannot be regarded as giving any real indication as to the degree of refinement or the ability of the oil to burn free from soot and smell and with a steady flame. Burning oils may be examined colorimetrically by Lovibond's tintometer, using the coloured glasses specially made for the purpose.

The most important tests to be carried out on burning oils are the distillation test, the specific gravity, the flash point, and the sulphuric acid test for the degree of refinement. Impurities such as sulphur and free acid should also be tested for.

Distillation Test.—This test may be carried out with the Engler apparatus, as described above for crude petroleum (p. 211). The temperature of the beginning of the distillation is taken when the first drop of distillate falls from the end of the condenser. Fractions are taken every 50° from 150° to 250°, and then every 25° up to 300°. What remains is estimated by difference. The end point for each fraction is the point at which after cooling at least 20° and reheating to the highest temperature at which the fraction is to be taken, not more than six drops of distillate run from the condenser, the process of cooling and heating being repeated till this result is obtained. The volume of each fraction is measured after cooling to the room temperature.

Distillation should not commence below 110°, the yield below 150° should be at most 10 per cent., and the yield above 300° at most 15 per cent. Better class oils yield 85 to 90 per cent. between 150° and 300°, and, at most, 5 per cent. above 300°. The higher the percentage of light oils, the greater the danger of explosion when the oil is burnt in a lamp, while the higher the percentage of high boiling constituents, the more liable will the oil be to clog in the wick and cause charring and uneven burning.

Specific Gravity.—The specific gravity of kerosene may be determined by any of the usual methods, as indicated under the heading of petroleum naphtha. The temperature correction per ° C. for reducing results to 15°, as determined by Mendeléeff, varies from 0·000790 for specific gravities between 0·760 and 0·780, to 0·000710 for specific gravities between 0·850 and 0·860. The specific gravity is mainly used as a test of identity, and is of little use, taken by itself; thus a burning oil might very well contain large proportions both of light and heavy oils and yet show a normal specific gravity; in a case like this, the inferior quality of the oil would only be revealed by the distillation test.

Flash Point.—The flash point of burning oils is usually determined in specially constructed "closed testers," which will be found described in the works of reference mentioned at the end of this chapter. The oil is gradually heated while a small flame is brought near its surface from time to time; the temperature at which the oil begins to give off sufficient inflammable vapour to produce a flash is taken as the flash point. The test thus gives an indication of the degree of safety with which the oil may be used in a lamp; the greater the proportion of lighter constituents, as shown by the distillation test, the lower will be the flash point and the greater the danger of explosion. In Great Britain and Canada the Abel tester has been adopted as the standard instrument, the minimum flash points, as laid down by the laws of these countries, being 73° F. and 85° F. respectively. In Germany and Russia, both the Abel and the Pensky-Martens instruments are used, the minimum flash point allowable being 21° C. (70° F.) in Germany, and 28° C. (84·4° F.) in Russia. In the United States various standard instruments and minima have been adopted in different states.

Degree of Refinement.—The following method for determining the degree of refinement of burning oils by the colour which they impart to concentrated sulphuric acid has been recommended by the Baku section of the Russian Technical Society. 100 parts by volume of the oil are shaken for two minutes in a glass-stoppered cylinder, with 40 parts by volume of sulphuric acid of specific gravity 1·73, at a temperature not exceeding 32°. The acid is then separated off and transferred to a cylinder of white glass, where it may be compared, as regards colour, with solutions of Bismarck brown, of known strengths, contained in similar cylinders. The layers of liquid observed should be of equal depth, and the cylinders should be placed on a uniform white surface. The test solutions are prepared as follows: A solution containing 0·5 gram of the colour per litre is first prepared, and from this ten solutions are made by dilution with water; the first and lightest contains 1 part of the stock solution to 99 parts of water, the second, 2 parts to 98 parts of water, and so on to the tenth and darkest, which contains 10 parts of the stock solution to 90 parts of water. These solutions are numbered from 1 to 10, passing from the lightest to the darkest, and the burning oil is marked according to the solution with which the sulphuric acid extract most nearly corresponds in tint.

As most petroleums are found to come within the limits of 1 to 8, the latter mark is taken to be the maximum limit for a marketable product.

Impurities.—Certain petroleums, notably those from Ohio and Canada, contain notable amounts of *sulphur compounds* which it is necessary to remove by special processes of refining, as kerosenes containing more than the slightest traces of such impurities will give a noticeable odour of sulphur dioxide on burning.

Owing to the relatively small amounts of sulphur present, even in the worst cases, special methods must be employed for the determination of this constituent. One of the methods most commonly employed is that of Allen, modified by Heussler and Engler, in which a known weight of the petroleum is burnt in a specially constructed lamp, and the products of combustion led over a solution of potassium hypobromite, which is distributed over glass beads. The sulphur is then estimated as sulphate in this solution. The above method is described in most of the works of reference mentioned at the end of this chapter. The Carius method may also be used.

According to Holde, a good burning oil should not contain more than 0·02 per cent. of sulphur.

Burning oils should be *free from acid*; about 20 grams of the oil dissolved in a neutral mixture of ether and alcohol containing a little phenol phthalein should show a permanent pink coloration on the addition of one drop of decinormal sodium hydroxide solution.

Ash may be determined, according to Holde, by the following method: 500 c.c. or a litre of the burning oil is distilled from a retort until 10 c.c. are left as still residue; the latter is transferred to a platinum dish, by the use of light petroleum, evaporated to dryness, and the residue incinerated.

Good burning oils should, according to Holde, contain not more than 2 milligrams of ash per litre.

THE EXAMINATION OF LUBRICATING OILS.

Most of the lubricating oils used nowadays are obtained from the residue which remains after distilling off the naphtha and burning oil from crude petroleum. This residue, which by itself boils above 300°, is distilled in a current of superheated steam at temperatures varying from 180° to 250°, whereby various fractions are obtained which show considerable differences in viscosity and flash point. Deodorisation is effected by blowing with air and treatment with strong sulphuric acid, the latter process being, as usual, followed by washing with caustic soda solution and water. Clarification is effected by filtering over fuller's earth or charcoal. The lighter lubricating oils, which vary in colour from light yellow to brown, and have specific gravities ranging from 0·895 to 0·900, are invariably obtained by distillation; the products of higher specific gravities, up to about 0·940, may consist either of "reduced oils," *i.e.*, filtered residues from which the more volatile portions have been removed by distillation, or of oils which have been distilled *in vacuo* or in a current of superheated steam. Undistilled products are generally used in the cheaper grades of lubricating oils.

Certain non-drying or semi-drying fatty oils are used as lubricants for delicate machinery, as, for example, neat's foot oil and spermaceti oil, which are used for watches and clocks. Generally, however, when fatty oils are used at all, they are used in admixture with mineral oils. Such blended oils generally contain rape or cotton seed oils which have sometimes been blown with air at elevated temperatures (see Chapter III., p. 130) in order to increase their viscosity. The detection and estimation of fatty oils in presence of mineral oils, and the reasons for their use, will be treated of below. For the present, it may be mentioned that the tendency for fatty oils to undergo hydrolysis and decomposition under the action of steam is greatly reduced when they are mixed with mineral oils. Rosin, rosin oil, coal tar or lignite oils must be considered as adulterants if found in lubricating oils purporting to be of superior grade. The detection of such constituents will be described below.

The requirements of a lubricating oil will naturally vary to a great extent with the conditions under which it is to be used. In general, however, it may be stated that lubricating oils should be free from acids, either mineral or organic, which may cause corrosion of the bearings, and also from all materials which may give rise to the "gumming" of the oil when spread in a thin layer, such as rosin, rosin oil or drying fatty oils. Water or sediment of any kind should be absent.

The function of a lubricating oil is to keep two metallic surfaces from actual contact with one another; for this it is necessary that it should possess a certain "body" or viscosity; the greater the pressure between the surfaces, the greater the viscosity required. On the other hand, the higher the viscosity of the oil, the greater its internal friction; it is, therefore, always advisable to use an oil of the minimum permissible viscosity in order to avoid excessive heating of the bearings. The temperature at which the oil is to be used must also be taken into consideration;. thus, the oils employed for the lubrication of steam cylinders are of a thick and syrupy consistency at the ordinary temperature, as the viscosity invariably decreases with rise of temperature. Fatty oils are sometimes used in cylinder oils, as they lose viscosity with rise in temperature to a less degree than the mineral oils. Oils which are to be used in refrigerating machines, or compressors, are quite mobile at the ordinary temperature, as the viscosity will increase on cooling. The viscosity of a lubricating oil, as determined by means of a viscometer, gives an indication of the class of work for which it is best suited, though in this case, practical experience should be the main guide. The Engler and Redwood viscometers are the standard instruments adopted for the testing of lubricating oils in Germany and the United Kingdom respectively. In the United States the Sayboldt instrument has been adopted.

Other physical properties which must be taken into account are the setting point and the flash point. The determination of these, and their significance in special cases, will be dealt with below.

Lubricating oils are classified by Holde as follows:—

(1) *Spindle Oils, for Textile Machinery.* Mobile oils

to be used under light pressures; viscosity in Engler degrees at 20° C., 5 to 12; flash point (Pensky), 160° to 200° C.

(2) *Compressor Oils*, or oils for use in refrigerating machines. Mobile oils having low viscosities; Engler degrees at 20° C., 5 to 7. It is important that these oils when submitted to the cold test should remain liquid at - 20°. The flash point may be low (Pensky), 170° to 180°. Compressor oils are often coloured a reddish violet.

(3) *Light Engine Oils*, suitable for motors or dynamos. These are moderately viscous; Engler degrees at 20° C., 13 to 25; flash point (Pensky), 170° to 220°.

(4) *Heavy Engine Oils.* Engler degrees at 20° C., 25 to 45, or in special cases up to 60; flash point (Pensky), 190° to 220°.

The foregoing oils, when viewed in a test tube, are light brownish yellow to brownish red in colour, the more expensive grades being light yellow. The following varieties are dark in colour and often opaque.

(5) *Dark Railroad Oils.* These are classified into summer and winter oils. The former show viscosities of 45° to 60° Engler at 20° C., and the latter 25° to 45°. The flash point (Pensky) should be over 140°. Setting point, for summer oils, under - 5°, for winter oils, under - 20°. (The above requirements for railroad oils are based on the climatic conditions of Germany; in England it is not necessary to prescribe so low a cold test in winter.)

(6) *Cylinder Oils.* These are of a syrupy or sometimes even pasty consistency at the ordinary temperature. The viscosity at 50° C. is 23° to 45° Engler. Oils for superheated steam cylinders have still higher viscosities,

i.e., 50° to 60° Engler at 50° C. The flash point should be over 200° and, according to the quality, varies from 220° to 315°; the better qualities flash above 260°. Low volatility is also a desideratum for cylinder oils; this generally follows with a high flash point. Cylinder oils which have been filtered over fuller's earth are of a brownish red colour and transparent; the unfiltered oils are greenish black and opaque.

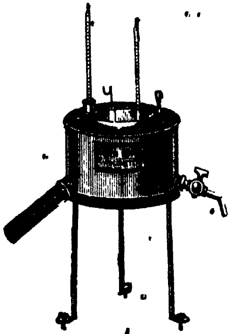

FIG. 16.
Redwood's Viscometer.

Viscosity.—By means of the viscometers mentioned above, the time taken for a definite volume of the oil (50 c.c.) to flow through an orifice of standard size under definite conditions is determined. As a rule, the efflux time is taken as being proportional to the viscosity of the oil, but as the flow is determined by gravity, the rate of flow will also be governed to some extent by the specific gravity of the oil. It is, of course, important to work at a definite temperature, and for the sake of uniformity and convenience in comparing results, it would be desirable if the suggestion of Fryer and Weston to make 40° and 100° the standard temperatures were adopted. For heavy oils which are intended for use at high temperatures, the determination at 100° is the more important, and for lighter oils that at 40° should be given chief consideration. Fryer and Weston further propose that results should be calculated to absolute viscosities multiplied by 100, which can be done if the instrument is calibrated according to Archbutt and Deeley's method, and to calculate the ratio of the viscosity at 40° to that at 100°, the viscosity ratio number thus obtained gives an indication of the loss of viscosity with rise of temperature, which varies with different oils and is an important factor in practice. The methods referred to may be found described in the works by Archbutt and Deeley, and Fryer and Weston, mentioned at the end of this chapter.

Flash Point.—The determination of flash point by the open test is carried out as follows: about 50 c.c. of the oil to be tested are placed in a porcelain or nickel crucible of about 75 c.c. capacity. The crucible is placed in a tin of convenient size to protect the surface of the oil from draughts, the bottom of which is covered with about half an inch of sand. The tin is heated from below, and the temperature of the oil is indicated by a thermometer, the bulb of which is immersed in the oil without touching the bottom of the crucible. When the temperature has been raised to about 120°, the oil is tested from time to time by bringing a small gas jet, burning from a hard glass capillary tube, near its surface. The lowest temperature at which the vapour from the oil is observed to ignite is taken as the flash point. When a flash has been obtained, the oil may be cooled about 10° and reheated at the rate of about 2° per minute to get a more accurate determination of the flash point. It should, however, be noted that the flash point will be sensibly raised if the oil is kept too long at an elevated temperature. The results obtained by the open test are not so accurate as those obtained by means of the closed testers, *e.g.*, the Abel or the Pensky-Martens instruments, but for practical purposes it is only necessary to be able to say whether the oil will be dangerous or not. The flash points indicated by the closed testers are usually about 15° lower than those obtained by the open test.

The determination of flash point is of especial importance in the case of oils which are to be used for textile machinery and oils for lubricating steam cylinders. If the oil has a high flash point, it is not only less liable to damage the rubber packings of the cylinders by ignition, but also less liable to loss by evaporation on prolonged exposure to elevated temperatures. The lighter oils for lubricating bearings and journals usually flash between 175° and 200° by the open test. The heavier machine oils should show flash points of 200° and upwards, while cylinder oils of good quality should flash above 280° by the open test. Considerable quantities of fatty oils are sometimes added to mineral cylinder oils of inferior quality in order to counterbalance their tendency to evaporate at the temperature of the steam cylinder, and their low flash points.

Setting Point.—The following method for the determination of the setting point of lubricating oils is recommended by the Scottish Mineral Oil Association. The oil is cooled in a large strong walled test tube until it completely solidifies. In the absence of liquid carbon dioxide or air, the following freezing mixtures may be employed: a mixture of 2 parts of snow or pounded ice with 1 part of sodium chloride, which gives a minimum temperature of - 23°, or a mixture of 12 parts of snow or pounded ice, 5 parts of sodium chloride and 5 parts of ammonium nitrate, which gives a minimum temperature of -30°. When the oil has solidified, the test tube is removed from the freezing mixture and held up to the light, while the oil is stirred with a thermometer; the temperature at which the last trace of paraffin disappears is taken as the setting point. The test is repeated until two determinations give concordant results.

As mentioned above, lubricating oils for refrigerating machines should remain liquid at -20°. The setting point, or, as it is sometimes called, the cold test, is also of importance in the case of lubricating oils for machinery which is used in the open in countries where the winter is severe; thus for some winter oils it is necessary to prescribe a setting point of -15° or -20°. In England, however, the cold test is of less importance. If the oil should solidify in the journals or bearings, friction would be developed with consequent damage to the machinery.

Specific Gravity.—In the case of the lighter oils, a float may be used; for thicker oils, it will be necessary to use a specific gravity bottle, as described for crude petroleum.

The specific gravity has no relation to the lubricating power of an oil, but mainly serves as a means of classification and identification. The specific gravities of pure mineral lubricating oils usually lie between 0·884 and 0·930 at 15°. The specific gravity cannot be looked on as proportional to the viscosity of the oil, but it may be said that, as a rule, oils of high specific gravities are used for high pressures.

Water.—Ordinary lubricating oils should contain no water. In light coloured oils water is easily detected by inspection on shaking the sample; in dark oils it will be revealed by the bumping or spitting which will take place on heating. If desired, any water which may be present may be estimated as described for crude petroleum.

Acidity.—The free acids liable to occur in lubricating oils may consist of sulphuric acid which has remained in the oil after the refining process, or organic acids arising from the decomposition of the mineral or vegetable oils. A high acidity may also be due to the presence of rosin or rosin oil, the detection of which will be described below.

Sulphuric acid may easily be detected by shaking out the oil with warm water, and testing the latter when separated, with barium chloride solution and hydrochloric acid; this impurity will, however, only be found in very exceptional cases.

The acidity of lubricating oils may be determined by titration as described under the heading "Acid Value" in Chapter III. If difficulty is experienced owing to the dark colour of an oil, the following process, recommended by Holde, may be employed: 20 c.c. of the oil are shaken in a glass stoppered measuring cylinder with 40 c.c. of neutral absolute alcohol, the oil being warmed if not sufficiently mobile. The cylinder is stoppered and allowed to stand overnight, after which 20 c.c. of the clear alcoholic layer are titrated with decinormal sodium hydroxide solution, using phenol phthalein as indicator. If more than 0·03 per cent. of acid (calculated to SO_3) is found, the rest of the alcohol should be poured off, the above process repeated with the residual oil and a further quantity of 40 c.c. of neutral alcohol, and the amount of acid found in this second titration added to that found in the first.

Refined, clear mineral oils should contain no free acid, or, at most, only 0·03 per cent., calculated as SO_3.

Dark oils should not contain more than 0·3 per cent. of acids; generally, however, the proportion of these constituents will be under 0·15 per cent. If the amount of free acids in a lubricating oil is above the prescribed limits, there is danger of the bearings on which it is used becoming corroded.

Deblooming Agents.—Nitronaphthalene is sometimes added in order to destroy or minimise the fluorescence of mineral oils. It may be detected by a reddish violet colour produced on boiling a few c.c. of the sample with alcoholic potash in a test tube, or by the odour of naphthalamine produced on heating with zinc dust and dilute hydrochloric acid.

Soap and Ash.—The better class lubricating oils will contain no soap or foreign inorganic matter, *e.g.*, talc or graphite, as will be shown by their complete solubility in benzene and low ash content. Soap may be added in order to increase the consistency of the oil or to facilitate its emulsification with water; it will be recognised by the ease with which emulsions are formed, and the feebly alkaline reaction of these towards phenol phthalein. An examination of the aqueous extract will soon show whether soap is present (see Chapters III. and IV., introductory sections), and the nature of the base (usually soda potash, lime or ammonia) may be ascertained by examining the acidified aqueous extract. The soap may be estimated as described for lubricating greases.

Other Tests.—A test for *gumming tendency* is described below.

Nastjukoff's *Formolite test*[1] gives a measure of the proportion of unsaturated cyclic hydrocarbons which react with formaldehyde and sulphuric acid to form solid condensation products to which the name "formolites" is given. 27 grams of the oil are dissolved in 50 c.c. of petroleum ether, and 30 c.c. of concentrated sulphuric acid are added without shaking. The mixture is cooled in ice water, and after the addition of 15 c.c. of 40 per cent. formaldehyde, it is shaken and cooled until no further heating takes place; it is then allowed to stand for half an hour, and poured into a litre flask containing 200 c.c. of ice water. The acid liquid is neutralised with ammonia, the precipitate is filtered off, washed with petroleum ether till free from oil, then with water till free from ammonia, and dried at 105° till constant in weight.

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[1] Marcusson's modification, Chem. Zeit., 1911, 35, 729 and 742, abs. *Analyst*, 1911, 461.

Marcusson found from 28·9 to 33·3 grams of formolite per 100 c.c. of American oils, and 10·4 to 24·4 grams per 100 c.c. of Russian oils. He states that the chief constituents to which mineral machine oils owe their lubricating power are those which do not yield formolites.

Loebell[1] describes a modification of Holde's method for determining *asphalt* in mineral lubricating oils. Kissling[2] has proposed a method for estimating tarry and carbonaceous matter in lubricating oils formed after heating at 150° to 250° for fifty hours.

LUBRICATING GREASES.

Soap is chiefly used in lubricating greases, in which it is emulsified with mineral, lignite, rosin or tar oils, with the addition of a little water. The soap may be estimated by carefully burning off a weighed quantity of the sample, incinerating and titrating the residual potassium carbonate, sodium carbonate, or lime, with seminormal hydrochloric acid. The amount of the base found may then be calculated to stearate or rosinate.

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[1] Petroleum, 1911, 6, 774, abs. *Analyst*, 1911, 418.

[2] Chem. Zeit., 1909, 33, 328, abs. *Analyst*, 1909, 328.

Bockermann[1] recommends the following method for the estimation of soap as being sufficiently accurate for technical purposes: After the estimation of the free fatty acids (as described for lubricating oils) 2 grams of the grease are boiled for thirty minutes with 50 c.c. of decinormal caustic potash solution and 50 c.c. of absolute alcohol in a flask under a reflux condenser. The mixture is cooled, and shaken with 40 c.c. of petroleum ether in a stoppered cylinder; when the layers have separated, 20 c.c of the petroleum ether are drawn off and evaporated; the residue of unsaponifiable matter is dried in an oil bath at about 140° till constant in weight. The soap is estimated by difference. If the amount of free fatty acids present is large, a corresponding correction must be made.

The oily matter may be separated from the soap for examination for the constituents mentioned above by repeating the operation on a larger scale; sufficient alkali should be used to ensure that no fatty acids find their way into the petroleum ether solution.

Water may be estimated in lubricating greases by the toluene distillation method, as described for crude petroleum.

DETECTION AND ESTIMATION OF FATTY OILS, ROSIN, ROSIN OIL, TAR OIL, ETC., IN PRESENCE OF MINERAL OILS.

Fatty Oils.—As a preliminary test, the saponification value of the sample may be determined as described in Chapter III., p. 131. If no definite value is obtained, then fatty oils may be assumed to be absent. The following method for the rapid determination of fatty oils in presence of mineral oils is recommended by Schreiber :—[1]

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[1] Chem. Zeit., 1911, 35, 1066, abs. *Analyst*, 1911, 559.

Five grams of the oil are weighed out in a 200 c.c. conical flask, 25 or 50 c.c. of approximately semi-normal alcoholic potash (see Chapter III., p. 132) are added, and then sufficient benzene to dissolve the oil when warmed. 25 c.c. of benzene will generally be sufficient, but for cylinder oils 50 c.c. may be necessary; in this case, 25 c.c. of neutral alcohol may be added with advantage. At the same time, a blank experiment is started with equal amounts of alcoholic potash, benzene and alcohol, to those used in the actual determination. The flasks are connected with air condensers and placed on a water bath so that the contents are kept gently simmering. During the saponification, which will be complete in thirty minutes, the contents of the flasks should be gently shaken from time to time. The amount of potash used up in the saponification is determined by titration with semi-normal acid in presence of phenol phthalein, as described under the heading "Saponification Value" in Chapter III. For practical purposes, the saponification value of the fatty oils used in lubricating oil may be taken as 195. Then, if S be the saponification value found, the percentage of fatty oil present will be

$\frac{100 S}{195}$. Rape and sperm oils, however, have saponification values of 175 and 124 respectively, and if either of these are suspected or found, due allowance must be made in the calculation.

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[1] *J. Amer. Chem. Soc.*, 1907, 27, 74.

The fatty acids may be obtained for examination with a view to possibly determining the nature of the fatty oil or fat present, by saponifying and extracting the unsaponifiable oil with ether as described on p. 237 (see also p. 193). The problem may be a difficult one, especially if mixtures of fatty oils and fats are present.

Lubricating Oils containing no Mineral Oils.—These may be recognised and examined by methods described in Chapter III. The fatty oils and fats which are used as lubricants are pointed out in the last column in the table on pp. 148 and 149, and mention is made regarding their use as blown or oxidised oils. As mentioned on p. 130, blown oils have lower iodine values than the normal oils; the refractive index will also be lowered on blowing, but the saponification value will not be greatly affected. Mention may be made of the enormous increase in the demand for *castor oil* during the last few years, owing to its particular suitability for aircraft engines.[1] The U.S. War Office specification for this oil is as follows: Must be colourless; specific gravity at 60° F., 0·959 to 0·968; must be completely soluble in four volumes of 90 per cent. alcohol of specific gravity 0·834 at 60° F.; maximum acidity not to exceed the equivalent of 1·5 per cent. of oleic acid; iodine value 80 to 90; saponification value 176 to 187; unsaponifiable matter under 1 per cent.; must contain no rosin, rosin oil or cotton-seed oil; viscosity (Sayboldt) 450 seconds at 130° F. or 95 seconds at 212° F.; flash point 450° F. in a Cleveland open flash cup; freezing point below zero F.

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[1] *J.S.C.J.*, 1919, 38, 20 R.

Rosin (see also Chapter IV.).—As was pointed out above, a somewhat high acidity may be due to the presence of dissolved rosin. In light coloured mineral oils, rosin may be detected by the Liebermann-Storch reaction, as described in Chapter IV., p. 189. If the oil be too dark, the rosin acids may be dissolved out by extracting with dilute sodium hydroxide solution and precipitated by acidifying the alkaline extract. This process may also be adapted for the quantitative estimation of rosin, provided that fatty oils are not present; the precipitated acids are filtered off, dried, weighed, after which they may be tested by the Liebermann-Storch reaction.

A quicker way of separating the rosin for qualitative examination is to shake about 10 c.c. of the oil with an equal volume of hot 70 per cent. alcohol, and after cooling, filtering and evaporating the alcoholic layer. If rosin be present, the residue will be of a resinous nature, and not oily, and it will give the characteristic coloration in the Liebermann-Storch test.

If fatty oils are present, it will be necessary to saponify as described under the heading "Fatty Oils" (p. 237). The alcohol and benzene having been removed by evaporation, the residual soap is dissolved in water, and the solution separated from unsaponifiable matter by extraction with ether. The soap solution is then evaporated to dryness, redissolved in water, and the solution acidified with hydrochloric acid; the precipitated acids are extracted with ether, after which the aqueous layer is separated off, neutralised and evaporated to about 25 c.c. On acidifying again and extracting with ether, the rest of the fatty acids will be separated. The ethereal extracts are united and evaporated; the residue may then be tested for rosin acids by the Liebermann-Storch reaction. If desired, the rosin acids may be estimated by Twitchell's process, as described in Chapter IV., p. 189, in the mixture of fatty and rosin acids obtained, as just described, from a given weight of the oil (sufficient to give about 5 grams of mixed acids). It should be noted that rosin acids which have been treated with hydrochloric acid, as in the Twitchell process, no longer give the Liebermann-Storch reaction.

Rosin Oil.—Rosin oil is obtained from rosin, or colophony, by destructive distillation. It always contains varying quantities, up to about 30 per cent., of unchanged rosin acids which have been carried over mechanically in the distillation, and may, therefore, be tested for in lubricating oils, as described above, by the Liebermann-Storch reaction. Rosin oil gives a violet coloration when shaken with a drop of stannic bromide (Allen).

Valenta's test for rosin oils, which has been investigated and recommended by Lewkowitsch, depends on the fact that 100 grams of glacial acetic acid at 50° dissolve only 2·6 to 6·5 grams of mineral oil but 16·9 grams of rosin oil. If fatty oils are present, they must first be removed by saponification, and the unsaponifiable matter isolated. Two c.c. of the unsaponifiable matter are mixed in a test tube with 10 c.c. of glacial acetic acid, and the tube is loosely corked and kept in a water bath at 50°, shaking continually for five minutes. The mixture is filtered through a moist filter, and the middle portion of the filtrate collected; a portion of this is weighed off accurately and titrated with normal caustic soda solution; the difference between the weight of acid and that found by titration represents the amount of oil dissolved.

Rosin oils are generally used in axle greases, together with soap and water, or as insulating material for electrical machines. Owing to their tendency to resinify when exposed to the air in thin layers at elevated temperatures, they are unsuitable for use in the better class lubricating oils. Rosin also has a tendency to cause gumming, and, like rosin oil, must be regarded as an adulterant if found to be present in better class lubricating oils.

Lubricating oils are sometimes tested for their *gumming tendency* by spreading them in thin layers on glass plates and keeping them at 50° or 100° C., the film being examined on cooling, from day to day. If rosin oil be compared in this way with a mineral lubricating oil of good quality, the superiority of the latter as regards gumming tendency may be shown.

Tar Oils.—The tar oils which are used for the adulteration of mineral lubricating oils are usually the heavy dark anthracene mother liquor oils (see Chapter II.), of specific gravity over 1·00. If present in fairly large quantity, therefore, they may sometimes be recognised by the somewhat high specific gravity of the sample. According to Holde, the following properties of tar oils enable them to be recognised in presence of mineral oils. They are completely soluble in alcohol at the ordinary temperature, giving dark-coloured solutions, and their smell is generally creosote like. When heated on the water bath with concentrated sulphuric acid, they react to form water soluble products. With nitric acid of specific gravity 1·45, they react vigorously, often with explosive violence, with the formation of nitro compounds. Coal tar oils are best detected and estimated by the use of dimethyl sulphate, which dissolves aromatic hydrocarbons in all proportions, but does not dissolve and is insoluble in the heavier paraffin hydrocarbons or rosin oils (Valenta). The oil is shaken with one and a half times its volume of dimethyl sulphate in a stoppered graduated cylinder, and the increase in volume of the dimethyl sulphate layer is noted. The test is accurate to within 1 or, at the most, 2 per cent. The coal tar oil may be isolated and weighed after saponifying the dimethyl sulphate by warming with dilute caustic soda solution. It should be noted that dimethyl sulphate is very poisonous.

Lignite Oils.—The lignite oils of high boiling point and specific gravity 0·9 to 0·97, which may be used for the adulteration of mineral oils, resemble the coal tar oils in having a creosote like smell; they are soluble in twice their volume of alcohol at the ordinary temperature, to the extent of 22 to 62 per cent. They contain unusually large amounts of sulphur compounds, and react with concentrated nitric acid less vigorously than the coal tar oils, but more vigorously than the mineral oils. They are not so easily recognised in mixtures as the coal tar oils.

Fuel Oils.—As explained above, these may be crude oils which do not contain any appreciable proportion of the more volatile constituents usually found in petroleums, or the residues remaining after distilling off the volatile matter (see p. 205). They may be analysed for water, and volatile constituents by the methods given above for petroleum, and the flash point, viscosity acidity and setting point may be determined as described for lubricating oils. The British Admiralty specification provides that the flash point shall not be lower than 175° F. (close test); the acidity must not be above 0·05 per cent. (as sulphuric acid); when tested in the

Redwood viscometer, the efflux time for 50 c.c. at 32° F. must not exceed 2000 seconds.

REFERENCES.

"Das Erdöl und Seine Verwandten," by H. von Höfer. 1912. Fr. Vieweg, Brunswick.

"Die Schmiermittel, Methoden zu Ihre Untersuchung und Wertbestimmung," by Grossmann. 1909. Kreidel's Verlag, Wiesbaden.

"The Chemical Technology and Analysis of Oils, Fats and Waxes," by J. Lewkowitsch. 3 vols. 1913-1915. Macmillan & Co., London.

"Das Erdöl," by Engler and Höfer. 3 vols. 1908 to 1911. S. Hirzel, Leipzig.

"Laboratoriumsbusch für der Erdölindustrie," by Kissling. 1908. Wilhelm Knapp, Halle.

"Handbook on Petroleum," by J. H. Thomson and Boverton Redwood. 1913. C. Griffin & Co., London.

Allen's "Commercial Organic Analysis" (revised edition), Vol. III. 1910. J. and A. Churchill, London.

"Chemisch-Technische Analyse," Vol. I., by Post. 1908. Fr. Vieweg, Brunswick.

"Lubricants and Lubrication," by Archbutt and Deeley. 1912. Chas. Griffin, London.

"The Examination of Hydrocarbon Oils and of Saponifiable Fats and Waxes," by Holde, trans. by Mueller. J. Wiley & Sons, New York; Chapman & Hall, London.

"The Examination of Lubricating Oils," by T. Stillmann. 1914. Chemical Publishing Co., Easton, Pa.

"Technical Handbook of Oils, Fats and Waxes," by Fryer and Weston. 1917 and 1919. Cambridge University Press.

"Liquid Fuels for Internal Combustion Engines," by H. Moore. 1918. D. van Nostrand, New York; Crosby Lockwood, London.

"Lubricating Oils, Fats and Greases," by Hurst, revised by Leask. 1911. Scott, Greenwood, London.

"Laboratory Book of Mineral Oil Analysis," by Hicks. 1919. Chas. Griffin, London.

"Oil Fuel," by Butler. 1914. Chas. Griffin, London.

CHAPTER VI

MILK AND BUTTER

INTRODUCTORY

ON account of the enormous value of the milk of the cow as an article of food, both in its original state and when manufactured into such products as butter or cheese, its complex nature and its susceptibility to decomposition through the agency of the manifold species of micro-organisms for which it forms an excellent nutrient medium, the examination of this product may well be said to form one of the most important and interesting chapters in the chemistry of foods.

Ordinary cow's milk of commerce is a white opaque emulsion of fat in water which contains lactose, saline matter and other substances in solution, and casein in a state of colloidal suspension. Its bluish or yellowish tinge depends on the amount of fat present, or on the presence of colouring matter, either natural or artificial.

Fresh milk has an amphoteric reaction, turning blue litmus slightly red, and red litmus slightly blue; this is owing to the presence of acid phosphates; on an average, 100 c.c. of fresh milk will be found to require about 30 c.c. of decinormal sodium hydroxide solution for neutralisation in presence of phenol phthalein, and 40 c.c. of decinormal sulphuric acid in presence of litmus as indicator. On standing, milk which has not previously been heated almost invariably becomes decidedly acid owing to the conversion of part of the lactose into lactic acid by certain micro-organisms.

The most important constituents of milk are water, fat, proteins, lactose and inorganic salts. The following data, showing the general variations in the composition of cow's milk, have been compiled by Fleischmann from many different sources :—

Water	86·5 to 89·5 per cent.
Fat	2·7 ,, 4·3 ,,
Proteins	3·0 ,, 4·0 ,,
Lactose	3·6 ,, 5·5 ,,
Ash	0·6 ,, 0·9 ,,

Richmond gives the following average composition based on numerous analyses of milk from different parts of England :—

Water	87·35 per cent.
Fat	3·74 ,,
Milk sugar	4·70 ,,
Casein..	3·00 ,,
Albumin	0·40 ,,
Ash	0·75 ,,
Other constituents	0·06 ,,

Fleischmann's results apply to mixed milk from herds; greater variations may be found when samples from individual cows are examined. Judging by English standards, the upper limit for fat in Fleischmann's table might be placed a little higher (see below).[1]

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[1] The composition of milk has been dealt with by Richmond in annual papers in the *Analyst* for a number of years. The results of examination of milk from farms received during 1914, 1915 and 1916, especially as regards variations in the fat percentages, have been dealt with by Arup, Huish and Richmond, *Analyst*, 1917, 119.

The composition of milk varies, not only with the age, state of health, and breed of the animal, but also with the district, climatic conditions, time of the year, general treatment, method of feeding and other factors.

Before proceeding to describe analytical methods, a short account will be given of the principal constituents of milk and butter.

THE CONSTITUENTS OF MILK.

Fat.—As stated above, this constituent is present in the form of an emulsion; the globules of fat generally measure from 0·0016 to 0·01 mm. in diameter, and number, on an average, 2½ to 3 millions per cubic millimetre. In milk, the fat is present in the liquid, supercooled state; on transformation into butter fat, it takes up the solid form which melts from 31° to 36°. This change is effected by thoroughly agitating cream which usually contains about 20 to 35 per cent. of fat, and which has usually previously been soured or "ripened" by lactic acid producing organisms, in specially constructed churns, at temperatures of about 10° to 15°; the solid fat coalesces and separates from the buttermilk in the form of small pellets, which, after washing with water, are worked by a process of kneading into butter containing some 12 to 16 per cent. of water. The composition of butter will be further treated of below.

When milk is allowed to stand at rest, the fat rises to the top, forming a layer of milk rich in fat, known as cream. In modern dairy practice, milk is separated in centrifugal machines from which it is possible to obtain cream containing anything up to 80 per cent. of fat at will; the skim milk obtained contains a few tenths per cent., or even less, of fat.

Milk-fat is peculiar in containing an unusually large number of different glycerides which include notable proportions of the glycerides of the lower fatty acids, especially butyric acid. As is pointed out in Chapter III., the latter circumstance is taken advantage of in the estimation of butter fat by the processes in which the volatile fatty acids obtained under certain conditions from a given weight of the fat are estimated. Besides the lower fatty acids, milk or butter fat yields on hydrolysis higher fatty acids, such as palmitic, stearic and oleic acids (see Chapter III., introductory), the acids of intermediate molecular weight being obtained in relatively small proportion. Milk fat is the most valuable fat known, and is, economically, the most important constituent of milk. The commercial valuation of milk is based very largely on its fat content, while from the fat percentage of the milk yielded by individual cows it is possible to calculate the amount of butter obtainable in relation to the fodder consumed by the animals.

Although the fat in milk from individual cows may vary from 1 to 8 per cent. when exceptional cases are included, the variations for mixed milk from herds of cows, taken all the year round, usually fall between 2·5 and 4·5 per cent. In England the fat percentage is at its maximum in November and December, and at its minimum in May and June when genuine samples may occasionally fall below the standard of 3·0 per cent. in fat content. In countries where the diet of the animals is more uniform throughout the whole year, the fat percentages are generally speaking subject to smaller variations. Evening milk is on an average 0·2 to 0·3 per cent. richer in fat than morning milk. In order to illustrate the influence of the breed of the animals, it may be mentioned that the milk from Jersey cows averages 5 per cent. of fat, while that from Dutch cows only averages 3·1 to 3·2 per cent. The standards adopted by different countries in fixing the minimum permissible proportion of fat will naturally vary to some extent. In the United Kingdom it is generally presumed that genuine milk should contain at least 3 per cent. of fat, and accordingly the Sale of Milk Regulations of 1901 provide that milk containing less than 3 per cent. of milk fat (or less than 8·5 per cent. of milk solids other than fat) is to be presumed not to be genuine, unless the contrary be proved. Thus, no absolute standard is fixed, but the burden of proof would lie with the defendant in the case of a prosecution.

Proteins.—The total proteins of milk amount, on an average, to about 3·5 per cent., of which about 2·9 per cent. is casein, and 0·6 per cent. milk albumin together with traces of milk globulin. Casein belongs to the phosphoproteins, but differs from other phosphorus-containing proteins in that it yields no xanthine and pyrimidine bases, or pentoses, on hydrolysis. Owing to the absence of carbohydrate groupings, it fails to give the Molisch reaction; this test (which may be carried out with egg albumen) consists in adding to an aqueous solution of the protein a few drops of an alcoholic solution of *a* naphthol and then concentrated sulphuric acid; if carbohydrate groupings are present, as is the case with egg albumen, a violet coloration will be formed at the surface of contact of the acid and aqueous layers.

On hydrolysis with baryta solution, casein yields, besides carbon dioxide and ammonia, a number of amino acids which consist, in the main, of monoamino fatty acids and cyclic derivatives of the latter. Pure casein is a white powder, insoluble in water and soluble in dilute alkali or acid solutions. It thus possesses feebly basic and acidic properties. In milk, casein exists in the form of a calcium salt, which, being present in the colloidal state, can only be filtered off by passing the milk through a filter of unglazed porcelain; in this way the total proteins of the milk may be obtained as a white mass.

When milk is treated with a small proportion of acetic or mineral acid, or allowed to become sour through the agency of lactic acid-producing organisms, it thickens owing to the precipitation of the casein which has been liberated from its calcium derivative. On warming the acidified solution, the casein coagulates, leaving a slightly turbid, yellowish whey, while on addition of excess of acid it is redissolved. On the addition of rennet, tannic acid, alcohol or inorganic salts, such as sodium chloride, copper sulphate, alum, etc., to milk, the casein is precipitated, in the form of a calcium derivative; in the case of precipitation by rennet a partial disruption of the protein molecule takes place. In this respect, casein resembles the majority of proteins, which may usually be separated and purified by the salting out of their aqueous solutions at various definite concentrations and temperatures. Commercial casein, which is usually precipitated by acid, is used for a variety of purposes; hardened by treatment with formaldehyde it can be transformed into a body which can be put to the same uses as celluloid, bone, etc.; it is used for glazing paper and in the manufacture of adhesive preparations, paints, enamels and edible preparations such as "Plasmon," "Sanatogen," etc.

Milk albumin is very similar in composition and properties to the albumin of the blood. It is not precipitated with the casein on the acidification of milk, and further differs from casein in not being salted out from neutral solution on saturation with magnesium or ammonium sulphate. It is, however, precipitated from slightly acid solution on saturation with the above salts.

Milk Sugar.—Milk sugar, or lactose, is a disaccharide which, on hydrolysis with dilute mineral acid, yields the hexoses galactose and dextrose in equal amounts. It reduces Fehling's solution, and is dextrorotatory in aqueous solution, the hydrated lactose $C_{12}H_{22}O_{11}, H_2O$ having a specific rotation of $[\alpha]_D + 52{\cdot}53°$ at 20° C. As will be shown below, the two last-mentioned properties are made use of in the quantitative estimation of lactose.

The fermentation changes undergone by lactose play an important part both in the souring of milk and the manufacture of butter from cream. Lactose is not fermented by the common yeasts, most of which attack cane sugar; by far the most important fermentation change which it undergoes is its transformation into lactic acid ($C_{12}H_{22}O_{11} + H_2O = 4\ C_3H_6O_3$).

This change is brought about through the agency of different species of micro-organisms which occur in milk, and is, under normal conditions, the first change in the decomposition of unheated milk by micro-organisms. As was pointed out above, the milk becomes coagulated owing to the precipitation of the casein by the acid. In modern dairy practice, cream is soured for butter-making by first pasteurising it, *i.e.*, heating to about 70° to 85°, in order to destroy most of the micro-organisms present, and then, after cooling to the ordinary temperature, infected with a culture of a lactic acid producing organism, such as *Streptococcus Lacticus*. By this means it is possible to obtain more uniform results and a better flavour in the butter than if, as in the old process, the cream were allowed to ripen spontaneously through the agency of such naturally occurring bacteria as may obtain a predominating influence. The formation of lactic acid is, quantitatively, the chief change which occurs in the souring of milk or cream; at the same time, however, traces of aroma-producing substances are formed which play an important part in the flavouring of the butter fat. In margarine manufacture, similar changes are produced in skim milk.

Mineral Matter.—The analysis of the ash of milk, allowing for the phosphorus contained in the proteins, and the carbonate formed from the organic matter on incineration, shows the mineral matter of milk to be composed as follows (Söldner, quoted from Barthel) :—

Sodium chloride	10·62
Potassium chloride	9·16
Mono potassium phosphate	12·77
Dipotassium phosphate	9·22
Potassium citrate	5·47
Dimagnesium phosphate	3·71
Magnesium citrate	4·05
Dicalcium phosphate	7·42
Tricalcium phosphate	8·90
Calcium citrate	23·55
Calcium combined with casein ..	5·13
Total	100·00

All the above constituents exist in the dissolved state, with the exception of the calcium combined with the casein, which exists in the colloidal state, and part of the calcium phosphate, which, although existing in the solid form, is exceedingly finely divided.

Other Constituents.—Lecithin, a glycero-phosphate of the trimethyl-ammonium base, choline, occurs, on an average, to the extent of about 0·065 per cent. in milk. The presence of this substance is said to cause the "browning" which takes place on heating butter.

Enzymes of different kinds are contained in milk; these include oxidases, reductases, catalases, and protein and fat hydrolysing enzymes. The significance of some of these enzymes, some of which are probably produced by the bacteria in the milk, will be dealt with later.

Dissolved gases, consisting of oxygen, carbon dioxide, etc.

Among *foreign bodies,* constantly occurring in milk, are white blood corpuscles or leucocytes, bacteria, yeasts, moulds and their spores and dirt.

The number of micro-organisms and amount of dirt contained in new milk depend on the cleanliness of the cows, the stable, and the vessels in which the milk is collected, as well as on the care which is taken in the subsequent treatment of the milk. Thus, while milk from clean cows, collected in steam-sterilised vessels, may contain only a few hundred bacteria per cubic centimetre immediately after milking, milk from dirty cows, collected in dirty vessels, may contain several hundred thousand bacteria per cubic centimetre. The dirt, which may consist of hairs of animals and human beings, earth, dung, fodder, fibres, sand, parts of insects, etc., may amount to as much as 0·03 to 0·25 per cent. of the milk, but should, in general, be below 0·01 per cent.

THE CONSTITUENTS OF BUTTER.

As will be gathered from the above remarks on the production of butter, the latter contains the constituents of milk in altered proportions. The percentage of fat in butter will generally lie between 83 and 85 per cent., water between 12 and 15 per cent., while the proteins generally amount to about 0·6 to 0·9 per cent., sugar and lactic acid to 0·4 per cent., and ash, *i.e.*, inorganic salts, to 0·1 or 0·2 per cent.

The fat, which differs from milk fat in being in the solid state, is present in the form of minute globules, emulsified in the aqueous serum which holds the proteins, or curd, in suspension and the other constituents, such as inorganic salts and sugar, in solution. The content of curd may vary somewhat according to the method of manufacture of the butter; thus, Storch gives the average protein content of butter from fresh cream as 0·64, and of butter from ripened or soured cream as 0·84 per cent. A certain amount of salt is almost invariably added to butter as a flavouring medium and as a preservative, in order to check the development of micro-organisms which may turn it rancid. The amount of salt added may vary from 1 to 5 per cent., the so-called fresh or mild cured butters containing less than 1½ per cent. Brine-pickled butters may contain as much as 6 to 9 per cent. of salt. Boric acid or borax preservative may be added in amounts up to one half per cent., expressed as boric acid.

THE PHYSICAL AND CHEMICAL EXAMINATION OF MILK CREAM AND SKIM MILK.

Sampling.—Owing to the tendency of the fat to collect at the top, the sample should always be taken after thoroughly mixing the bulk of the milk. The sample bottle and cork should be perfectly clean, and the bottle should not be filled more than about three-quarters full, in order that the cream may be mixed into the bulk of the milk by shaking, immediately before examination.

If the sample is required for bacteriological analysis, it should be taken in a bottle which has not only been well cleaned, but sterilised by heating in an oven to 150° for half an hour, the stopper and neck having been wrapped round with cotton wool which is kept in place by a piece of muslin tied over it.

If the analysis cannot be proceeded with at once, the sample is best preserved by keeping it in a cool place, preferably in an ice chest. The addition of 0·5 gram of potassium dichromate, or 1 c.c. of a 40 per cent. solution of formaldehyde per litre, will preserve milk for a prolonged period; it is, however, advisable to avoid, as far as possible, tampering with the sample in any way; further, the addition of dichromate will naturally affect the specific gravity and content of ash and solids less fat, while the addition of formaldehyde may interfere with the determination of fat by centrifugal methods.

Immediately before the analysis the sample bottle should be repeatedly turned in order to mix the cream with the rest of the milk, heating, if necessary, to about 40°. The milk is then poured into a clean beaker; if any cream remains in the bottle, the milk should be returned to it and shaken until only a trace of cream adheres to the sides. Every time a portion of the sample is to be weighed or measured off, it should be mixed by pouring from one vessel to another. In case the sample for analysis should be soured and coagulated, it may be treated with ammonia as recommended by Weibull;

Richmond's procedure, which consists in adding 5 c.c. of dilute ammonia (one part of strong ammonia to four parts of water), may conveniently be adopted; the consequent correction of the specific gravity is described below. If the sample should contain lumps of churned fat (resembling butter), which may occur if it has been shaken too much in warm weather, it should be strained through muslin, the lumps being analysed separately for fat.

PHYSICAL EXAMINATION.

The analytical processes described under this heading include the determination of specific gravity and dirt or foreign matter. In appearance, the milk should be fully opaque and homogeneous, and on standing at rest, it should form a well-defined layer of cream; a collection of flaky particles indicates either udder disease or that the milk is so old that bacterial decomposition has set in. Skim milk will, of course, separate no cream layer, and in milk which has been heated, the separation is slower and less pronounced according to the temperature to which the milk has been heated. A pink colour will in all probability be due to blood owing to local damage to the udder. In the presence of blood a red deposit will be obtained on centrifuging, which may be examined under the microscope for blood corpuscles. A sour taste or smell indicates that lactic acid-producing organisms have become active, while a bitter or saltish taste indicates either that the milk is derived from cows which have been improperly fed or are suffering from udder disease, or else that the proteins of the milk are being decomposed by bacteria with the formation of peptones. A metallic taste is generally due to the use of untinned vessels, while the injudicious use of certain fodders, such as mangel-wurzels or turnips, also produces characteristic tastes in the milk. According to modern investigations, it would appear that the last mentioned tastes originate from substances and bacteria introduced with the cow dung, so that the objections raised against these root fodders are not wholly justified. Milk very easily acquires the taste and smell of materials, such as carbolic acid or dung, if left in their neighbourhood for some time.

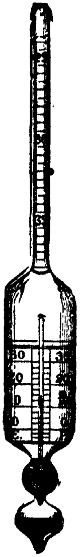

FIG. 17. Soxhlet's Lactometer.

Specific Gravity.—The specific gravity of milk is determined with the object of detecting adulteration, either by addition of water or removal of cream ; the interpretation of the results will be left to a subsequent section. (See " Solids less Fat.")

The determination should not be made within a period of three hours of the milking, owing to the slight increase in specific gravity which takes place at first, probably owing to some change in the colloidal state of the casein (Recknagel's phenomenon). The determination may be carried out in the usual way by means of the Westphal balance, specific gravity bottle, or, as is more commonly the case, with a float specially designed for the purpose, known as a lactometer. Soxhlet's lactometer is sometimes furnished with a thermometer, and is graduated to show specific gravities from 1·024 to 1·038. The length of the scale divisions is so adjusted that the fourth decimal place may be estimated. The instrument is adjusted to 15° C., the specific gravity found indicating the ratio of a given weight of the milk to that of an equal weight of water at this temperature. Readings may be reduced to 15° by adding two units to the fourth decimal place for each degree above 15°, and subtracting two units for each degree below; these corrections hold good for temperatures between 10° and 20° C. It is not essential that the lactometer should be provided with a thermometer, in fact, most lactometers are made without; the temperature correction should, of course, never be omitted. Vieth's lactometer is similar to Soxhlet's, but stouter and shorter. It is always advisable to check the instrument, and determine any correction which may be necessary by comparison with one which is known to be correct, or with the Westphal balance or pyknometer. In checking, careful note should be taken of the position of the graduation read relative to the surface of the milk (see below).

Before taking the specific gravity the sample should be well mixed and then poured into a cylinder of suitable size, avoiding the formation of froth on the surface. The float is carefully lowered into the milk until the division 1·025 coincides with the surface, and then allowed to come to equilibrium of its own accord. Keeping the eye on a level with the surface of the milk, the reading is taken at the highest point at which the milk is seen to rise up the spindle. The specific gravity of whole milk usually lies between 1·029 and 1·034, or, as is sometimes stated, between 29° and 34°. Skim milk has a somewhat higher specific gravity, usually between 1·035 and 1·037, owing to the absence of fat.

If ammonia has been added according to Richmond's direction (see under "Sampling"), an equal proportion of the same ammonia should be added to fresh milk, and the change in specific gravity thus occasioned noted; the correction may then be applied to the sour sample.

Dirt and Foreign Matter.—The following method by which the foreign insoluble matter is estimated in milk is due to Stutzer: the apparatus required consists of a bottle of about a litre capacity, having a tapering neck, connected by means of a wide piece of rubber tube to a strong test tube without a rim. The bottle is charged with a litre of milk, connected with the test tube, and the whole is allowed to stand for two hours in an inverted position, so that any sediment will collect in the test tube. The rubber connection is then pinched together with a clip, and the bottle is disconnected; the milk is decanted from the sediment in the test tube, which is washed several times with distilled water acidified with a little hydrochloric acid, in order to dissolve the calcium phosphate, which is a normal constituent ot the milk. The sediment is collected on a Gooch crucible, washed with water until the filtrate is no longer opalescent, then with alcohol and ether, dried at 100° and weighed. It may further be examined under the microscope.

Berch recommends that the milk should be preserved with formalin (see under "Sampling"), in order that it may be allowed to settle for twenty-four hours.

Good clean milk should contain only from 5 to 10 milligrams of dirt per litre, though milk of commerce often contains considerably more. The figures obtained by this method should not be taken too literally, as some of the original dirt may have dissolved in the milk, some adheres to the fat globules and is carried to the surface, while a further quantity may dissolve in the alcohol and ether. Thus, the principal solid impurity of milk is cow dung, and this contains a large proportion of water and soluble matter. The bacteria introduced with the dung will therefore readily become distributed throughout the milk. The method, however, gives an idea of the care which has been bestowed on the important operation of straining the milk. Quite recently a conviction was obtained for an excessive amount of dirt in milk.

A method by which the relative number of micro-organisms may be roughly estimated with a view to forming an opinion as to the care and cleanliness in the previous treatment of the milk is described later.

CHEMICAL EXAMINATION.

Under this heading will be described the determination of the acidity of milk, fat in whole milk, skim milk and cream, proteins, including casein and albumin, lactose and ash in milk, after which the analytical results will be discussed from the point of view of the detection of the adulteration of milk. Finally, tests for the pasteurising and bacterial contents of milk will be dealt with. The examination of milk, as well as butter, for preservatives will be considered in Chapter VIII.

Acidity.—This is determined by titrating with standard caustic soda, baryta, strontia or lime solution, using phenol phthalein as indicator. Very often the degree of acidity is understood to express the number of c.c. of normal caustic alkali solution required to neutralise 1000 c.c. of milk; Soxhlet and Henkel, however, use one-quarter normal alkali per 100 c.c., and their degrees, which are often used on the continent, are thus two-fifths of the above degrees. Richmond titrates 10 c.c. of milk with decinormal baryta solution, or 11 c.c. with eleventh normal strontia, using 1 c.c. of ½ per cent. phenol phthalein; as a standard, he uses an equal volume of milk coloured with one drop of 0·01 per cent. rosaniline acetate in 96 per cent. alcohol. In practice it is often found convenient to titrate 20 c.c. of milk with decinormal caustic soda; the alkali solution should be kept in a bottle provided with a soda lime guard tube, the bottle being placed above the burette so that the latter can be filled automatically. The burette may also be provided with a soda lime tube if it is filled through a side tube, or a small burette which is frequently emptied may be used. Care should be taken always to use the same amount of indicator and to titrate to the same tint.

Normal fresh milk which has an amphoteric reaction towards litmus generally shows an acidity of 16 to 19 degrees when tested by the above method, *i.e.*, ten times the number of c.c. decinormal alkali per 10 c.c. An acidity below 15 will indicate that the milk has probably been derived from sick cows or that it has been diluted with water; the reaction towards litmus will then be alkaline. A high acidity in milk which is known to be fresh will also indicate that it is abnormal. Normal unheated milk almost invariably becomes sour on keeping owing to the action of the lactic acid bacteria which convert the milk sugar, or lactose, into lactic acid. It is interesting to note that these organisms which are usually regarded as being the most characteristic of the milk bacteria, owing to the fact that they readily obtain predominance in the first stage of bacterial decomposition, are derived from the manure, and always become established in the milk by indirect if not by direct infection. Although it is useful to be able to detect incipient souring in a sample, bacterial multiplication will always be far advanced at this stage; in this respect, the reductase test (see p. 288) gives far sharper indications. Acid formation proceeds until an acidity of over 100 has been reached, corresponding roughly to about 1 per cent. of lactic acid; if the milk is kept at temperatures from 40° to 50°, over double the amount of acid may be produced owing to the action of the bacteria usually associated with Bulgarian sour milk, *e.g.*, *Bacillus Bulgaricus* (named *Thermobacterium Bulgaricum* by Orla Jensen). The well-known phenomenon of curdling, apart from the action of rennet or bacteria which produce rennet-like enzymes, is due to the precipitation of the casein by the lactic acid formed by bacteria.

When the acidity reaches the neighbourhood of 25 degrees, the milk will generally coagulate on boiling, and at an acidity of about 21 it will generally coagulate on the addition of an equal volume of 68 per cent. alcohol. The empirical "boiling" and "alcohol" tests have been based on the above properties.

Bacterial souring is of industrial importance in the souring of cream for butter making and skim milk for margarine making. The organism generally employed for these purposes is that known as *Streptococcus Lacticus* named *S. Cremoris* by Orla Jensen. Properly soured cream is more easily churned than fresh cream; it also gives a better yield of butter, and a more uniform and less perishable product. The acidity aimed at in the case of cream will naturally depend on its fat content.

Special milk preparations, such as Bulgarian sour milk, Koumiss, Danish "thick milk," etc., are produced wholly or partly by the action of lactic acid bacteria; these organisms also play an important part in the making and ripening of many cheeses, for example, *Thermobacterium Helveticum* both in the making and ripening of Gruyère cheese.

Fat.—A number of methods have been devised for the estimation of this very important constituent. They may be divided into two classes: first, the accurate methods, such as the Gottlieb, Werner-Schmidt, Adams, Babcock asbestos and maceration methods, and, second, the Gerber, Babcock, and Leffmann and Beam methods, which are less accurate but more rapid and adapted for the testing of large numbers of samples. Owing to the importance of the estimation, several methods will be described, but it will only be possible to make a selection of some of those in use.

ACCURATE METHODS FOR THE DETERMINATION OF FAT IN MILK AND CREAM, ETC.

The Gottlieb Method.—This method was formerly looked on with disfavour by some workers in this country, but it is an excellent method if properly carried out. Barthel and Weibull recommend it as being better than any other for skim milk and butter milk (see Barthel's work mentioned at the end of this chapter). Mooi and Partridge recommend it for sour milk, and Richmond recommends it for dried milk and general use. Various special pieces of apparatus have been designed for carrying out the Gottlieb method; the best known of these is the Farnsteiner tube; Richmond's modification is described here, as it only requires a tall narrow stoppered cylinder of about 50 c.c. capacity.

Five c.c. of the milk are carefully measured into the cylinder by means of a pipette, the exact weight of milk delivered by the pipette under standard conditions having been determined. Half a c.c. of ammonia (strong ammonia, specific gravity 0·880, diluted with an equal volume of water) is added and the mixture is well shaken; 5 c.c. of alcohol are added and the mixture is shaken; the solution of any lumps present is facilitated by warming in hot water. 12·5 c.c. of ether are added and the mixture is shaken well; 12·5 c.c. of petroleum ether, boiling below 60°, are then added and the shaking is repeated; after standing for a few minutes, the tube is again shaken. The success of the operation will depend on the addition of the ingredients in the right order and shaking well each time. As soon as separation of the layers is complete, which will be in about five to ten minutes, the ethereal solution is removed as completely as possible to a 200 c.c. flask by means of a wash bottle arrangement made of the smallest possible quill tubing; if the delivery tube is lengthened so as to form a syphon, less disturbance of the layers is likely to take place. The mixture is then shaken with three successive portions of a mixture of equal volumes of ether and petroleum ether (the recovered solvent may be used), which are transferred to the same flask. After most of the solvent has been distilled off, the flask is placed in the water oven and dried to a constant weight. After weighing, the fat is just melted and extracted from the flask by washing with four successive portions of 5 c.c. of petroleum ether, and the flask is dried for half an hour in the water oven and weighed. The difference between the two weights gives the weight of the fat. The method of estimating the fat in an aliquot portion of the ethereal solution, obtained by using a graduated tube, has been found by Richmond to be less trustworthy than that described.[1]

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[1] *Analyst*, 1908, 389.

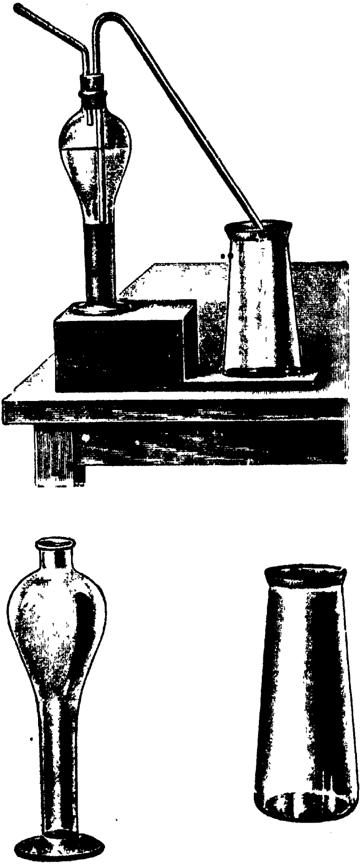

FIG. 18.—Eichloff's Apparatus for the Gottlieb Process.

Cream may be treated in the same way, 1 to 1½ grams being weighed into the tube from a small beaker and then made up to 5 c.c. with water.

Dried Milk.—0·5 to 0·7 gram is placed in the tube and made up to 5·15 grams with water; after adding the ammonia and alcohol as described above, thorough solution should be ensured, if necessary, by warming.

Eichloff[1] has designed the modified form of apparatus shown in Fig. 18 for carrying out the determination of fat according to the Gottlieb process. The vase-shaped vessel possesses obvious advantages over the long graduated tube which it is designed to replace, being of such size and weight that it can conveniently be weighed on the balance; the milk or cream may, therefore, be directly weighed out in it. As there are no graduations, the whole of the fat must be weighed; it is, however, only necessary, after the first potion of the fat solution has been syphoned off as completely as possible, to wash out the vessel with two successive portions of 25 c.c. of ether, which need not be shaken with the ammoniacal alcohol. The drying of the fat is considerably facilitated by using the specially designed beaker flask shown in the figure, instead of an ordinary flask. After the solvent has been evaporated off, the fat may be completely dried within one hour, in this way, errors owing to oxidation are avoided. As in the original process, double the quantities of milk and solvents mentioned above are used, but otherwise the details of manipulation are the same.

The Werner-Schmidt Method.—This is similar in principle to the foregoing method, but the milk (10 grams) is boiled with an equal bulk of hydrochloric acid till dark brown instead of being shaken with ammonia and alcohol, while ether is used to extract the fat. The Stokes tube used for this method is a graduated tube which has a constricted middle portion. If the whole of the fat is extracted, 30 c.c. of dry ether are added in the first instance (after cooling), and this is followed by three successive portions of 20 c.c. of ether. Richmond prefers the method of adding 50 c.c. of ether and drawing off an aliquot portion as the error due to the solubility of lactic acid in ether is increased by repeated extractions. The fat is estimated as in the foregoing method. The tendency for small quantities of substances other than fat to be extracted with the fat is somewhat greater than in the foregoing method.

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[1] *Milchwirtschaftliches Centralblatt*, 1910, p. 114.

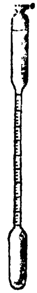

FIG. 19.—Stokes' Tube for Werner Schmidt Method.

The Adams Method.—This is the oldest of the methods in use at present. Five c.c. of the milk are carefully distributed from a pipette delivering a known weight under the same conditions on to a piece of fat-free blotting paper specially made for the purpose. The paper is allowed to dry spontaneously in a place free from flies, warmed for a few minutes in the water oven, then coiled up and placed in a Soxhlet extractor, where it is extracted for five hours with ether which has been washed with water and dried by calcium chloride. General directions for extractions and illustrations of the apparatus are given on pp. 87 to 89. Care should be taken to exclude moisture, or a little of the milk sugar may be rendered soluble. The ether is distilled off, and the fat dried to constant weight in the water oven. It is pointed out by Richmond that the method gives low results with homogenised milk, *i.e.*, milk in which the fat globules have been artificially comminuted.

The Maceration Method.—This method is more laborious than the foregoing ones, but it is specially applicable to sour and decomposed milk, and is used by the referees under the Sale of Food and Drugs Act in the Government laboratory.[1]

Babcock's Asbestos Method.—This method has been adopted by the American Association of Official Agricultural Chemists. A hollow cylinder of perforated sheet metal, 60 mm. long and 20 mm. in diameter, closed 5 mm. from one end by a disc of the same material, is required. The perforations should be about 0·7 mm. in diameter and about 0·7 mm. apart. The cylinder is filled loosely with 1·5 to 2·5 grams of freshly ignited woolly asbestos free from fine and brittle material. After cooling in a desiccator the cylinder with asbestos is weighed; a weighed quantity of 3 to 5 grams of the milk is soaked up into the asbestos, and the whole is dried to constant weight at 100°; the *total solids* are thus determined. The fat is extracted by dry ether in an extractor, and may be weighed after distilling off the ether or by difference. Most of the working details are similar to those given under the Adams method.

Calculation Method.—This is not so accurate as the foregoing direct methods. It depends on a knowledge of the percentage of total solids and the specific gravity, and the application of the formula connecting these values with the fat percentage. (See p. 279.)

Rapid Methods for the Determination of Fat in Milk and Cream.—In the methods which are most commonly employed in practice, the fat is separated from the other constituents of the milk or cream by the use of the centrifuge, and its volume read off.

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[1] For descriptions of this method, see Richmond's "Dairy Chemistry," also Thorpe, *Analyst*, 1905, 197, and Richmond and Miller, *Analyst*, 1906, 317.

Gerber's Method.—Fig. 20 shows one form of butyrometer tube or bottle which may be used for the analysis of milk by this process. Into this tube are introduced 10 c.c. of concentrated sulphuric acid of specific gravity 1·820 to 1·825 at 15° C., and 11 c.c. of the milk, taken from a well-mixed sample, are carefully run in from a pipette so as to avoid complete admixture with the acid. Finally, 1 c.c. of amyl alcohol of specific gravity 0.815 at 15° C. and boiling point between 128° and 130° is added. The acid and alcohol which are employed to facilitate the separation of the fat in a clear layer are added from measures which automatically deliver the required amounts, or from burettes or pipettes. A well-fitting long rubber stopper is inserted in the butyrometer tube, which is then well shaken till all lumps are dissolved and whirled in a centrifuge at about 1,000 revolutions per minute for three to four minutes. The tube and its contents are brought to 65° in a water bath, and the fat percentage is directly read off by observing the graduation to which the lowest part of the meniscus of the fat layer reaches, the lower surface having been brought to the zero division, or any

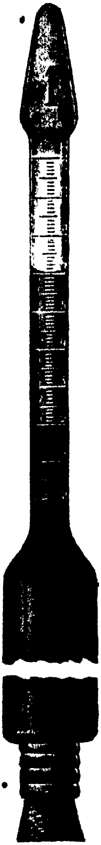

FIG. 20.
Gerber Tube for Whole Milk.

other convenient mark, by manipulating the rubber stopper.

Similar tubes may be obtained for the analysis of skim milk or cream by this method. The tubes for whole milk are usually graduated to show up to 7 or 9 per cent. of fat, while those for cream show up to about 40 or 60 per cent. of fat, using either 5 grams or 5 c.c. of cream. The special tubes, measures, and all other requisites for the above process, may be obtained from most dealers in chemical apparatus.

The Gerber bottles and bottles used in similar methods should be checked by duplicate determinations made by one of the more accurate methods. The layer read as fat is by no means pure fat; the method is subject to several errors which tend to balance one another. Richmond has made a thorough study of the Gerber method, including the calibration of the bottles.[1]

The Babcock Method.—This method is used in America in place of the Gerber method which it closely resembles.[2] Fig. 22 shows the type of bottle used. 17·6 c.c. of the milk are first run into the bottle from a pipette, and then 17·5 c.c.

FIG. 21. Gerber Tube for Skim Milk.

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[1] "Dairy Chemistry" and *Analyst*, 1905, 325, and 1918, 405.

[2] "Chemical Testing of Milk and Cream," U.S. Bureau of Animal Industry, 1917.

of sulphuric acid of specific gravity 1·82 to 1·83. The acid and the milk should be run down the side of the neck. The mixture is shaken without interruption until solution is complete, and the bottle is centrifuged for four to five minutes. Hot water is then added until the contents come nearly to the bottom of the neck, and the whirling is repeated for a minute. More hot water is added to bring the fat to a point below the top graduation mark in the neck, and the whirling again repeated for a minute. The percentage of fat is read off from the graduations, the temperature of the contents being at a temperature between 130° and 140° F., the temperature of the added water being regulated so that the fat will be read at such a temperature. The first portion of hot water is to be run down the side of the neck, the second to be dropped through the fat to clear it. The fat reading is taken from the lowest point of the meniscus.

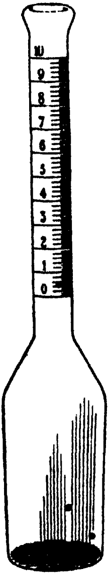

FIG. 22.
Babcock Bottle for Milk Testing.

The Leffmann and Beam Method.—This is a modification of the Babcock method; Leffmann and Beam originally introduced the use of amyl alcohol in order to facilitate the separation of the fat and thus to reduce the time of whirling. The Gerber method was based on this process. Bottles similar in shape to the Babcock bottles are used; these are graduated for use with 15 c.c. of milk. Three c.c. of a mixture of equal parts of amyl alcohol and hydrochloric acid are added and shaken with the milk. Nine c.c. of sulphuric acid (96 per cent., specific gravity 1·842 at 15·5°) are carefully added and mixed with the contents so as to avoid sudden overheating and boiling. The surface of the liquid is brought near the top graduation mark by adding a hot mixture of equal parts of sulphuric acid and water, the bottle is whirled for one or two minutes, and the clear fat layer is read off from the bottom of the meniscus.

Notes on the Centrifugal Methods.—Of the above methods, the Gerber is the simplest and most convenient. The various bottles may occasionally be incorrectly graduated and should be tested against bottles known to be correct, or one of the more accurate methods; in the latter case the divergence should not be more than about 0·15 per cent. The bottles should be cleaned immediately after use by rinsing with hot water several times. The sulphuric acid must be of the correct strength or failure will result. The amyl alcohol should be submitted to a blank test, using in the Gerber method 10 c.c. of acid, 10 c.c. of water, and 2 c.c. of the alcohol. No "fat reading" should be obtained, and, if this is the case, the alcohol will contain petroleum which will vitiate the results.

A large number of determinations may be rapidly carried out by the methods involving centrifuging, the results obtained being sufficiently accurate for ordinary technical and commercial work.

Proteins.—The method of estimation of the total proteins of milk by treating 10 grams of milk according to the Kjeldahl process for estimating nitrogen, and calculating the percentage of nitrogen thus found to proteins, by multiplying by 6·37, does not give very reliable results, as the whole of the nitrogen contained in the milk is not present as proteins. Better results are obtained by estimating separately the casein and albumin by the methods now to be described.

Casein.—The determination of this constituent must be carried out on fresh, or nearly fresh, uncoagulated milk. If the analysis cannot be proceeded with during the next twenty-four hours, the sample should be preserved as described under the heading of "Sampling," preferably by keeping it in a refrigerator, but failing this, by means of potassium dichromate.

The methods by which casein is separated from albumin and other constituents of milk depend on the fact that the casein is salted out, *i.e.*, precipitated, by the addition of solutions of certain salts such as alum or magnesium sulphate, while the albumin is not so precipitated, excepting in the presence of free acid. After the casein has been salted out the albumin may be precipitated by the addition of tannic acid, with which it forms, like most of the proteins, an insoluble compound; it may also be precipitated from the saturated magnesium sulphate solution by boiling with a little dilute acetic acid. Both the casein and the albumin are estimated as nitrogen by the Kjeldahl process.[1] An alternative method consists in precipitating the casein by adding a little dilute acetic acid to the diluted milk and estimating the albumin in the filtrate by the Kjeldahl process. This method is, however, not so reliable as the others indicated above, as the casein is not always quantitatively precipitated by means of acid.

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[1] The Kjeldahl process is described on pp 20 to 27. For the present purpose it is only necessary to use sulphuric acid and copper oxide or sulphate as the conversion to ammonia is effected fairly easily.

The American Association of Official Agricultural Chemists recommend the following method for the estimation of casein in milk:—

To 5 grams of milk add 50 c.c. of a solution of magnesium sulphate saturated at 45°, and heat the mixture to 45° till the precipitate separates and subsides, leaving the supernatant liquor clear. Collect the precipitate on a filter, wash two or three times with a solution of magnesium sulphate, as used above, at the same temperature, *i.e.*, 45°, and, after drying in the steam oven, transfer the filter and precipitate to a Kjeldahl digestion flask for the determination of the nitrogen. The amount of nitrogen found, multiplied by 6·25, gives the weight of casein present in the sample analysed.

An alternative method for precipitating casein (Schlossman) is to dilute 10 c.c. of milk with 40 c.c. of water, and to add, while stirring, 1 c.c. of a cold saturated solution of alum, when the casein quickly separates. If the supernatant liquid is not quite clear a further quantity of alum solution, not exceeding 0·5 c.c., is added, drop by drop. The casein is then filtered off and estimated as nitrogen by the Kjeldahl process.

For the precipitation of the casein by means of acid, the following method is recommended by the American Official Association of Agricultural Chemists:—

Ten grams of milk contained in a beaker are diluted with 90 c.c. of water at 40° to 42°, and 1·5 c.c. of a 10 per cent. solution of acetic acid in water is added at once. The mixture is stirred with a glass rod and allowed to stand for five minutes, after which the supernatant liquid is poured off, and the precipitate washed with cold water by decantation. The casein is collected on a filter and estimated as nitrogen by the Kjeldahl process.

Albumin.—The filtrate with washings obtained by any of the above methods, from the precipitated casein, is treated with 10 c.c. of a solution of 4 grams of tannic acid in 8 c.c. of 25 per cent. acetic acid, which has been made up to 200 c.c. with 40 to 50 per cent. alcohol; the resulting precipitate is filtered off after settling, washed, dried and analysed by the Kjeldahl process. The amount of nitrogen found, multiplied by 6·34, gives the amount of albumin (and globulin) present in the milk.

It was stated above that albumin is only precipitated from a saturated solution of magnesium sulphate in presence of acid. If the casein has been precipitated by means of this salt, as in the first of the methods described above, the albumin may be precipitated by neutralising the filtrate from the casein with dilute sodium hydroxide solution, adding 0·3 c.c. of 10 per cent. acetic acid and heating in boiling water for ten to fifteen minutes. The precipitate is collected on a filter and analysed for nitrogen, as in the previous method.

It is, of course, necessary that the filter paper used for collecting the precipitated proteins should be either free from nitrogen or else of known nitrogen content, as it is treated together with the proteins in the Kjeldahl determination. The tannic acid used should also be tested to make sure that it is free from nitrogen.

Total Proteins by "Aldehyde Figure."—The following rapid method is Richmond's modification of Steinegger's process.[1] It depends on the fact that proteins combine with formaldehyde to form acidic substances which can be estimated by titration. To 10 or 11 c.c. of milk at least 1 c.c. of ½ per cent. phenol phthalein solution is added, and the milk neutralised with eleventh normal (approximately) strontia solution. Two c.c. of 40 per cent. formaldehyde solution are added, and the titration continued until the same tint of pink is restored. The number of c.c. used in the second titration, multiplied by ten to bring it to the acidity degree basis, is known as the aldehyde figure or amino acid number. For strontia, this number, multiplied, by 0·170, gives the percentage of proteins in the milk. The acidity of the formaldehyde must be determined and deducted from the result of the second titration. For decinormal soda the factor is 0·191. In the case of abnormal milks the method breaks down, and if the milk is sour, high results are obtained owing to the proteins having been partially hydrolysed and thus presenting more amino groups to react with the formaldehyde. Richmond, however, found the method to be reasonably accurate so long as the milk was not curdled. It may be pointed out that the above factors are only applicable to the proteins of normal cow's milk.

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[1] *Analyst*, 1908, 115, and 1911, 9.

Milk Sugar.—Two methods will be described for the determination of this constituent in milk, the one gravimetric and the other optical. In this connection Chapter VIII. should be consulted as well.

Gravimetric Method.—The process to be described, due to Allihn and Soxhlet, depends on the ability of lactose to reduce a warm alkaline solution of a cupric salt (Fehling's solution), the amount of the insoluble cuprous oxide formed under given conditions being taken as a measure of the lactose present. The amount of lactose corresponding to a given weight of copper or copper oxide, as obtained by the method to be described, is determined by reference to the accompanying table, as the amount of cuprous oxide precipitated is not strictly proportional to the amount of lactose present. Before the actual estimation of the lactose in milk can be proceeded with, the proteins must be removed as follows (Ritthausen) :—

Twenty-five c.c. of milk are weighed out and diluted with 400 c.c. of water in a 500 c.c. flask. The proteins are precipitated by adding 10 c.c. of Fehling's copper solution (containing 69·28 grams of $CuSO_4$, $5H_2O$ per litre, *not* the alkaline tartrate solution), and then

SOXHLET'S TABLE FOR FINDING THE WEIGHT OF LACTOSE CORRESPONDING TO A GIVEN WEIGHT OF COPPER REDUCED ACCORDING TO ALLIHN AND SOXHLET'S METHOD OF DETERMINING LACTOSE

Cu, milligrams.	Lactose, milligrams.	Cu, milligrams.	Lactose, milligrams.	Cu, milligrams.	Lactose, milligrams.
140	101·3	240	176·9	340	255·8
145	105·1	245	180·9	345	259·8
150	108·8	250	184·8	350	263·9
155	112·6	255	188·7	355	268·0
160	116·4	260	192·6	360	272·1
165	120·2	265	196·4	365	276·3
170	123·9	270	200·3	370	280·5
175	127·8	275	204·3	375	284·8
180	131·6	280	208·3	380	289·1
185	135·4	285	212·3	385	293·3
190	139·2	290	216·3	390	297·7
195	143·1	295	220·3	395	302·0
200	146·9	300	224·4	400	306·3
205	150·7	305	228·3		
210	154·4	310	232·1		
215	158·2	315	236·0		
220	161·9	320	239·9		
225	165·6	325	243·8		
230	169·4	330	247·7		
235	173·1	335	251·7		

Factor for converting CuO to Cu = 0·7989.

sufficient decinormal sodium hydroxide solution to give a neutral or feebly acid reaction. Usually, from 6·5 to 7·5 c.c. of the alkali solution will be sufficient; in no case should enough be added to produce an alkaline reaction. 20 c.c. of a cold saturated solution of sodium fluoride are then added in order to remove the dissolved calcium salts, the presence of which tends to influence the results of the sugar determination. After allowing to settle for half an hour the flask is filled to the 500 c.c. graduation, and the clear solution filtered through a dry filter. In a deep porcelain dish, 50 c.c. of Fehling's solution (made by mixing 25 c.c. of the copper sulphate solution, as used previously, with 25 c.c. of a solution containing 250 grams of potassium hydroxide and 350 grams of Rochelle salt per litre) are heated to boiling, and 100 c.c. of the filtrate containing the lactose, obtained as just described, are added. After stirring, the mixture is boiled for exactly six minutes, and the precipitate formed is filtered off on a Gooch crucible, washed with water, alcohol and ether, and converted into cupric oxide by placing the Gooch crucible inside an ordinary crucible and heating over a Bunsen flame. The amount of lactose may then be found by reference to the table. In Allihn and Soxhlet's original method the cuprous oxide was reduced to copper and weighed as such in a special tube.

For a polarimetric method, see Chapter VIII., p. 391.

Total Solids. Direct Determination.—About 2½ grams of the milk are weighed as quickly as possible in a shallow porcelain dish 2½ to 3 inches wide (milk dish). The dish is then heated on the water bath until the residue appears dry; in order to prevent the formation of a skin, Revis' procedure of adding 1 c.c. of acetone may be adopted. The dish and residue is then transferred to a water oven and weighed every hour. It is not possible to dry to an absolutely constant weight, for the residue decomposes slightly on prolonged heating, as is indicated by its turning brown. The weight may be taken as constant when the loss is not more than a milligram per hour. For rapid routine work, when a number of samples have to be tested, the milk may be measured from a pipette graduated to deliver 5 grams of milk of specific gravity 1·0320. The time of drying on the water bath and in the oven will be standardised, so that repeated weighings are unnecessary.

Cream may be analysed in a similar manner; Richmond advises the mixing of the cream with its own volume of alcohol before drying, and when the residue is apparently dry, to incline the dish so that the fat does not cover the non-fatty residue.

The Babcock Asbestos Method.—This has already been described in connection with the estimation of fat. The method is recommended by Richmond, who uses it for the determination of total solids alone by drying the milk on asbestos contained in a crucible.

Calculation Method.—If the specific gravity and fat percentage are known, the total solids may be calculated by Richmond's formula:—

$$\text{T.S.} = G/4 + 1{\cdot}2\,F + 0{\cdot}14,$$

where G = the lactometer degrees, *e.g.*, for specific gravity 1·0321, G = 32·1, and F = fat percentage.

In routine analyses the total solids and the specific gravity may be determined, and the fat percentage calculated, or the fat and specific gravity determined and the total solids calculated. The usefulness and reliability of the above formula have been established by numerous analyses. Tables based on it are used in routine practice. Other formulae have been elaborated by Fleischmann and Babcock.

Solids not Fat.—These are very simply found by subtracting the fat from the total solids. They should always be calculated, as they are subject to much smaller variations in genuine milk than the total solids.

Ash.—Twenty grams of milk or less are weighed into a platinum dish and evaporated to dryness on the water bath, with the addition of a few drops of acetic acid or alcohol to prevent the formation of a skin. The residue is carbonised at a clear red heat, cooled and extracted with water. The aqueous extract is filtered, taking care that as little as possible of the carbonaceous matter is brought on to the filter. The remaining carbon, which has now been freed from most of the mineral matter, is burnt to ash, after which the aqueous extract containing the mineral matter is returned to the dish and evaporated to dryness. The whole is then heated at a low red heat until a perfectly white ash is obtained, cooled and weighed.

A simpler method is to ignite the total solid residue from 5 grams of milk over a Bunsen burner till a white ash is obtained. To prevent undue volatilisation of the salts, a scarcely perceptible red heat should be employed; under these conditions the loss is very slight.

Other Constituents.—For the determination of the other constituents of milk, such as citric acid and lecithin, see the works mentioned at the end of this chapter.

THE DETERMINATION OF THE GENUINENESS OF MILK.

The Sale of Milk Regulations (1901) made by the Board of Agriculture provide that "Where a sample of milk (not being milk sold as skimmed, or separated, or condensed milk) contains less than 3 per cent. of milk fat, it shall be presumed for the purposes of the Sale of Food and Drugs Acts, 1875 to 1899, until the contrary be proved, that the milk is not genuine by reason of the abstraction therefrom of milk fat or the addition thereto of water," and "Where a sample of milk . . . contains less than 8·5 per cent. of milk solids other than milk fat, it shall be presumed . . . that the milk is not genuine by reason of the abstraction therefrom of milk solids other than milk fat, or the addition thereto of water." The Sale of Milk Regulations (1912) similarly places the minimum of solids not fat for skimmed or separated milk at 8·7 per cent.

Another form of adulteration leading to a deficiency in fat, not mentioned above, is the addition of skimmed milk, which, however, practically comes to the same thing as abstracting cream.

As mentioned above, samples below standard as regards fat percentage are most likely to occur during the spring and early summer. Deficiency in solids not fat, may occur during the months of July, August, and September. If it can be proved that no adulteration has taken place, milk not conforming to the standards will not be considered as having been sold "to the prejudice of the purchaser." In certain cases, as when the farmer feeds his cows for quantity regardless of quality, or partially milks his cows (the richest milk is obtained towards the end of the milking and thus reserved for the calves), the present state of affairs is hardly satisfactory to the consumer.

In calculating percentage deficiencies or amounts of added water, the above minima must be taken as standards in the absence of a duly authenticated sample from the same source. In this way the figures obtained will often be below the truth, and it is therefore the custom to qualify the figures with the words " at least."

Deficiency in Fat is calculated as follows : Supposing the sample to contain 2·5 per cent. of fat, then the actual deficiency must be assumed to be 0·5 per cent., and the percentage deficiency will be at least $\frac{0\cdot5 \times 100}{3} = 16\cdot7$ per cent.

If the Milk has been watered, the solids less fat will be lowered ; suppose the solids less fat to be 8·1 per cent. The actual deficiency is assumed to be 0·4 per cent., and the amount of added water will be at least $\frac{0\cdot4 \times 100}{8\cdot5} = 11\cdot8$ per cent.

Watering will, in the first place, be indicated by a low specific gravity ; if this is under 1·0300, the sample must be regarded with suspicion, but the real criterion is the percentage of solids less fat, as a low specific gravity may be due to a high percentage of fat.

If the sample is not too old, the amount of added water may be confirmed by the percentage of proteins as determined by the formaldehyde titration (see p. 275), assuming the normal percentage of proteins to be 3·4.

Deficiency in Fat owing to removal of Cream or addition of Skim Milk will not be accompanied by a lowering of the specific gravity and the percentage of solids not fat ; on the contrary, these values will be slightly raised, for the specific gravity of separated milk containing only about 0·1 per cent. of fat is usually about 1·035, and the percentage of solids not fat about 9·0. If the fat percentage was reduced to 3·0 by such means, the analyst would not be able to supply any evidence of sophistication from an examination of the sample, though anyone detected in the act would be liable to prosecution for not supplying the milk as it came from the cow.

Normal and Abnormal Milk.—Vieth found that the proportion of lactose to proteins to ash in normal milk is as 13 to 9 to 2, and Richmond has confirmed this ratio. In abnormal milks the ash content is often high and the milk sugar low. Many abnormal milks are low in solids not fat.

THE HEAT STERILISING AND PASTEURISING OF MILK. THE BACTERIA OF MILK.

It is a well-known fact that all micro-organisms are destroyed when exposed to a sufficiently high temperature. On this principle it is possible to improve the keeping powers of milk by temporarily heating it to a suitable temperature. It was shown by Pasteur that temperatures lower than 100° could be employed with success in destroying most of the micro-organisms of milk, and Weigmann has shown that momentary heating to 85° is sufficient to destroy the most resistant of the pathogenic bacteria which are likely to occur in milk. Some bacteria, yeasts and moulds, however, produce highly resistant spores which are only destroyed by a heating to 115° to 120° for a quarter to half an hour. Milk is sometimes sterilised in this way in bottles; it may then often keep for years, but it has a decidedly "cooked" flavour. Continuous or "flash" processes for pasteurising in which the milk is heated to a definite temperature for about half a minute, are not very satisfactory for ordinary milk of commerce, for it is hardly possible to submit the milk to a temperature sufficiently high for efficient pasteurisation without giving it a slightly cooked flavour. On the other hand, cream or separated milk for butter, margarine, or biscuit making may with advantage be pasteurised by the flash process at 80° to 85°, for any pathogenic bacteria which may be present will thus be destroyed. The importance of the proper pasteurisation of cream for butter-making will be appreciated in view of the fact that most of the tubercle bacilli in milk tend to pass into the cream on separating; as it is possible to determine whether milk has been heated to at least 80° or not (see p. 287), the Danish pasteurisation law requires that cream for butter-making, skim milk, and butter milk (the two last mentioned products are largely used for cattle feeding in Denmark) shall be heated to at least 80°.

It should, however, be noted that if pasteurised milk is kept too long, the changes which it will undergo, owing to the action of micro-organisms, will have much more serious consequences than in the case of unpasteurised milk, especially if it was not cooled thoroughly immediately after heating. The reasons for this are as follows: In ordinary milk, kept at the ordinary temperature, the relatively harmless lactic acid producing bacteria develop at a far more rapid rate than all the other types of organisms present, and thus effectively keep the latter in check. In this first stage of decomposition, the milk is protected against putrefactive decomposition leading to the production of poisonous substances, while some time will always elapse before sufficient free lactic acid has been produced to coagulate the milk; even when this has occurred, however, the milk will not contain any poisonous substances resulting from the souring process. The lactic acid produced effectively checks the development of the protein hydrolysing, putrefactive bacteria which only become active in neutral or feebly alkaline media, and it is only after the souring process has come to an end (*i.e.*, when the milk contains about 1 per cent. of lactic acid), and the lactic acid has been consumed by the yeasts or moulds which usually follow after the lactic acid producing organisms have ceased to develop, that the putrefactive organisms are able to develop and act on the milk.

When the milk has been pasteurised at a high temperature, the conditions are entirely altered ; the lactic acid organisms are destroyed and leave no spores which might survive the elevated temperature. Many putrefactive organisms (*e.g.*, *B. subtilis*, or the hay bacillus), on the other hand, form highly resisting spores which survive the pasteurising process and, on cooling, are able to develop in the absence of lactic acid bacteria. The first stage in the decomposition of pasteurised milk is, therefore, often due to the development of putrefactive organisms which hydrolyse the proteins of the milk into the bitter poisonous peptones, and further into polypeptides, amino acids and amines.

For these reasons, the " holder " process of pasteurisation has come into favour during recent years, especially in America. This process involves the heating of the milk for longer periods at lower temperatures, at which some of the lactic acid bacteria survive. Ayers and Johnston[1] have found that on heating milk for half an hour to 63°, the percentage of lactic acid bacteria among the surviving organisms is greater than it was among the original organisms, but that at temperatures above 76·7° the lactic acid organisms are mostly destroyed, the majority of the bacteria which survive being of the peptonising type.

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[1] " A Study of the Bacteria which survive Pasteurisation," U.S. Dept. of Agriculture, Bureau of Animal Industry, Bull. 161.

Although pasteurisation is a useful process when properly carried out, it must not be looked on as a process for saving milk which has begun to deteriorate, for objectionable bacteria and bacterial products may have been formed which it will be impossible to eliminate on heating.

The effect of pasteurising on milk may be readily shown as follows: Several clean tubes of about 200 c.c. capacity are plugged at the mouth with cotton-wool, and sterilised by heating to about 140° in an air oven, for about twenty minutes. They are then allowed to cool, without removing the cotton-wool plugs, and nearly filled with fresh milk, the plugs being replaced as soon as possible after the introduction of the milk. One tube is kept unheated, a second may be heated to 63° for ten minutes in a water bath, and a third to 80°. During heating the milk is kept stirred by a thermometer by which the temperature is taken, and, after heating, the tubes are cooled as soon as possible in running water. The samples may then be examined by the reductase and fermenting tests as described below, the remainders of them being kept in the plugged tubes at the ordinary temperature. The acidity and the taste of the samples may then be compared after standing for a day or more, when it will be found that the unheated milk goes sour much quicker than the heated samples, but that the sample heated to 80° ultimately develops the most unpleasant taste and smell owing to peptonisation. The foregoing remarks will also be borne out by the results of the fermenting test, though it must be mentioned that good milk containing few bacteria may give a bad result in this test owing to the fewness of the lactic acid bacteria present. Even if this be so, the sample heated to 63° will probably yield a gelatinous coagulum (see above). The destruction of most of the bacteria by heating will be indicated by the increased reduction times.

Test for Pasteurising.—The following test is that devised by Storch. None of the numerous modifications which have been proposed from time to time can be said to be improvements on the original test. Milk contains an enzyme known as peroxidase, which has the property of causing hydrogen peroxide to oxidise certain organic substances; the visible effect with paraphenylene diamine is a coloration which appears blue in the presence of milk casein. This enzyme is partly destroyed on heating to just below 80°, and wholly destroyed on heating to 80°. It is not destroyed on heating at lower temperatures, even if the heating be prolonged for half an hour, so that the test is of no use for detecting pasteurisation carried out at low temperatures.

The test is carried out as follows: To 5 c.c. of the milk or cream in a test tube add 1 drop of a 0·2 per cent. aqueous solution of hydrogen peroxide, shake, then add 2 drops of a fresh 2 per cent. aqueous solution of paraphenylene diamine, and mix again by shaking. If the milk has not been heated, the nascent oxygen from the hydrogen peroxide will act on the paraphenylene diamine, giving rise to a blue coloration within a few seconds. If the milk has been heated above 80°, no colour will be developed in thirty seconds, while if it has only been heated to 78° to 80° a greyish blue colour will form in about thirty seconds. This test may easily be verified.

Reductase Test.—The test which is now to be described affords a means of ascertaining the relative number of micro-organisms present in milk. It has already been pointed out that the alteration in flavour, and decomposition of milk, are due to the action of the numerous species of micro-organisms which grow and reproduce in it. It is obvious that the greater the number of micro-organisms present, the quicker will be the decomposition of the milk. Milk as it leaves the udder of a healthy cow contains relatively few organisms, all of which are generally of a harmless nature. From this moment onwards, however, it is liable to become infected by organisms from the air, or any dirt, fodder, straw, etc., with which it may come into contact. Unless, therefore, the greatest care and cleanliness be observed in the treatment of the milk, its keeping powers will be materially reduced.

The reductase test depends on the reduction of methylene blue to a colourless substance, the time required for the reduction of a certain quantity of the dye by a given volume of milk being noted. Although the mechanism of the process is not perfectly understood, Barthel and Orla Jensen[1] have shown that the time of reduction as determined by the method given below affords a means of classifying milk according to its bacterial contents.

Test tubes of slightly over 40 c.c. capacity are required, these should preferably be strongly made and marked at 40 c.c. They should be well cleaned and rinsed in boiling water before use. The methylene blue solution may be made from tabloids specially prepared by Messrs. Blauenfeldt & Tvede of Copenhagen. A solution containing

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[1] *Milchwirtschaftliches Centralblatt*, 1912, 14.

0·07 per cent. of pure methylene blue may be used; the strength of the methylene blue may be determined as described by Knecht and Hibbert.[1] Forty c.c. of the milk are introduced into the tube, and 1 c.c. of the methylene blue solution is added and mixed with the milk by inverting the tube several times, closing it with the palm of the hand, which must be quite clean. When all the tubes are ready they are placed in a water bath which is kept at 38° to 40°, and the time for complete decolorisation noted in each case. Barthel and Orla Jensen found that in the majority of cases milk might be classified as follows:—

	Containing Organisms per c.c.
Class 1. Decolorised in five and a half hours or longer - - -	Under ½ million.
Class 2. Decolorised in less than five and a half hours, not less than two hours - - - -	½ to 4 millions.
Class 3. Decolorised in less than two hours, not less than twenty minutes - - - - - -	4 to 20 millions.
Class 4. Decolorised in less than twenty minutes - - -	Over 20 millions.

The results were checked with reference to gelatine plate counts, which, however, do not always afford an infallible means of estimating the number of micro-organisms. The reductase test has been examined by Arup,[2] who found that such discrepancies as do occur between the results of plate counts and the reductase test judged by the above scheme may be minimised by carrying out the test at 28° to 29°. This, however, precludes the test from being combined with the fermenting test described below, but, on the other hand, the latter test is the less important of the two for general purposes. From the practical point of view, the results of the test were what might be expected, judging from the conditions of working on the farms inspected.

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[1] "New Reduction Methods in Volumetric Analysis," 1918, Macmillan.

[2] *Analyst*, 1918, 1.

Fermenting Test.—Peter's fermenting test is mainly of importance in judging the suitability of milk for cheese making. Orla Jensen proposes to combine it with the reductase test, the type of fermentation being noted after twelve hours. The types are as follows: (1) The gelatinous type due to the predominance of lactic acid forming bacteria; the curd may be quite homogeneous or broken up to a slight extent by gas bubbles. (2) The gassy type due to predominance of putrefactive bacteria; the curd is more or less broken up by gas bubbles and almost invariably partly decomposed into soluble products owing to the peptonising action of these organisms. A disagreeable smell will be noticed, and sometimes the smell of butyric acid will predominate. This is the worst type, and the gelatinous type is the best. (3) The curdy or spongy type arises when the gas bubbles are very minute; this type is almost as objectionable as the gassy type. (4) The cheesy type is due to the predominance of bacteria which secrete rennet-like enzymes; the curd contracts and clear whey is produced. This type is less objectionable than Nos. 2 and 3.

In judging milk according to the results of the reductase and the fermenting tests, the results of the latter are not taken into account when the milk is placed in classes 1 or 4 according to the former.

The above remarks regarding pasteurising may be verified by submitting raw milk and milk pasteurised at different temperatures to the reductase and fermenting tests.

CONDENSED MILK

Condensed milk is manufactured by evaporating fresh milk *in vacuo* at temperatures of about 40° to 50° to a quarter or a third of its original bulk. Cane sugar is often added as a preservative to prevent the development of micro-organisms, so that the product may keep indefinitely in closed vessels. If sugar is not added, it is necessary to sterilise the condensed milk after tinning. When condensed milk is diluted with three to four times its bulk of water, it gives a product which has the same composition and general properties as new milk, excepting for the presence of cane sugar or invert sugar, and a cooked flavour.

Before taking the sample for analysis, the contents of the tin should be well mixed by stirring. After diluting in accordance with the instructions on the tin, the determination of the various constituents may, in some cases, be proceeded with as in the case of ordinary milk, and the results calculated back to percentages on the original condensed milk; in the presence of cane or invert sugar, special methods, to be described in Chapter VIII., must be adopted for the estimation of the carbohydrates. An unsugared product may be diluted to a specific gravity of 1·032 at 15° in the case of whole milk, or to 1·036 in the case of skim milk. If cane or invert sugar be present, the specific gravity can neither be used as an index for diluting the condensed milk to a strength corresponding to that of ordinary milk, nor for the calculation of the solids less fat as described above.

In the following table will be found typical analyses of different varieties of condensed whole and skim milk, by Buttenberg and others (quoted from W. Grimmer) :—

—	With Cane Sugar.	Without Cane Sugar.		Condensed Skim Milk.	
		Evaporated to One-Half.	Evaporated to One-Third.	With Cane Sugar.	Without Cane Sugar.
Water . . .	27·88	76·70	66·91	27·43	69·0
Fat . .	9·62	6·80	9·75	0·29	0·30
Proteins . .	10·27	5·89	8·95	11·59	12·4
Lactose . . .	14·20	9·13	12·50	13·60	15·7
Cane sugar . .	36·06	-	—	44·92	-
Ash . . .	1·97	1·48	1·89	2·17	2·6

Carbohydrates.—Besides lactose and cane sugar, or sucrose, it is possible that the condensed milk may contain invert sugar, *i.e.*, the mixture of dextrose and laevulose obtained by the hydrolysis of sucrose.

The lactose is estimated in the diluted sample as described above for ordinary milk. As has already been mentioned, sucrose does not itself reduce Fehling's solution, and its presence will therefore not affect the determination of the lactose by the gravimetric method. Invert sugar, on the other hand, reduces Fehling's solution, and will, if present, be determined together with the lactose, in which case it will be necessary to determine the latter separately by other methods which will be described in Chapter VIII.

The total sugar content may be approximately estimated by subtracting the sum of the fat, proteins and ash, determined in the diluted sample, as described above for ordinary milk, from the total solids, determined directly by evaporation, also as described above. Subtracting the percentage of lactose from that of the total sugar, the content of sucrose will be found with sufficient accuracy for most purposes. If greater accuracy is desired, the sucrose must be determined separately, as described below.

If the amount of reducing sugar found would correspond to a larger proportion of lactose relatively to the proteins and ash in the sample than would be expected in ordinary milk, then the presence of invert sugar may be suspected. If, on the other hand, the amount of reducing sugar found corresponds with a normal amount of lactose in proportion to the proteins and ash in the sample, but is appreciably less in amount than the total sugar, then sucrose may be assumed to be present.

Qualitative Test for Sucrose.—Sucrose in condensed milk may be detected by the following method, due to Gayaux: 10 c.c. of milk (or 0·5 gram of lactose to be tested for sucrose, dissolved in 10 c.c. of water) are warmed in a test tube with 50 milligrams of resorcin and 0·5 c.c. of 25 per cent. hydrochloric acid. The presence of sucrose is indicated by a red coloration after boiling for a few minutes, no colour being developed if lactose alone is present. According to Richmond, 0·2 per cent. of cane sugar may be detected by this test.

Calculation of the Composition of the Original Milk, and the Degree of Concentration of the Condensed Milk.—The fat contained in the original milk before evaporation may be approximately calculated from the non-fatty solids in the condensed milk, exclusive of cane sugar, invert sugar, or other added material. For the purposes of the calculation, the percentage of non-fatty solids in ordinary milk may be assumed to be 8·9.

Then, the percentage of fat in the original milk =

$$\frac{\text{Fat in condensed milk} \times 8{\cdot}9}{\text{Solids less fat in condensed milk}}.$$

Or, on the basis of the protein content of the condensed milk, assuming the protein content of ordinary milk to be 3·4 per cent., the percentage of fat in the original milk =

$$\frac{\text{Fat in condensed milk} \times 3{\cdot}4}{\text{Proteins in condensed milk}}.$$

In this way it is possible to determine whether the condensed milk has been made from whole milk, or milk which has been deprived of its fat, either wholly or in part. The degree of concentration of the condensed milk may be arrived at by a simple calculation from the solids less fat or proteins contained therein, assuming the percentages of these constituents in the original milk to have been 8·9 and 3·4 respectively.

Milk Powder and Infants' Foods.—Milk powder, or dried milk, is generally produced from whole, partially skimmed, or completely separated milk either by evaporating the condensed milk by passing it over heated rollers or by spraying it in a very finely divided condition into a chamber in which hot air circulates. The former process gives a flaky product, while the latter gives a fine powder which is more readily soluble in water. As regards convenience in handling and storing, dried milk possesses great advantages over condensed milk; although the product is not entirely sterilised in the process of drying, whole milk powder keeps well in sealed tins, and separated milk powder in wooden boxes or barrels, if stored in a dry place. The milk fat, being in a fine state of division, is liable to deteriorate if exposed too much to air, a circumstance which is said to render the spraying process unsuitable for treating whole milk. Dried milk has been very favourably reported on as a food for infants by many authorities. For this and the other reasons stated above, it is likely to become better known in the future. The dietetic value, manufacture, bacteriology, etc., of dried milk is fully discussed in a recent Report to the Local Government Board.[1]

The following table will give a general idea of the composition of dried milk :—

	Per Cent. in Whole Milk Powder.	Per Cent. in Separated Milk Powder
Moisture	2 to 6	3 to 10
Fat	23 to 31	0·5 (or more)
Proteins	23 to 26	30 to 37
Lactose (hydrated) .	34 to 41	46 to 52
Ash	5·5 to 7·5	7·0 to 9·5

Cane sugar or sucrose has sometimes been found in dried milk, usually in small amounts up to about 3 per cent. The estimation of sucrose in presence of lactose is described in Chapter VIII.

Some infants' foods on the market consist almost entirely of dried milk ; small amounts of sodium bicarbonate may be added to increase the solubility, sodium citrate to increase the digestibility of the curd, while varying amounts of lactose may be added to simulate human milk. The analysis of infants' foods containing a starchy basis is described in Chapter VII.

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[1] Reports to the Local Govt. Bd. 1. " Upon an Inquiry as to Dried Milks, with Special Reference to their use in Infant Feeding," by Coutts (with Appendices). 2. " Some investigations bearing on the Nutritive Value of Dried Milk," by Winfield. 3. " On the Examination of Milk Powders at the Government Laboratory." These three reports are bound together as Food Reports, No. 24.

Solution in Water.—Milk powder may be "dissolved" by mixing first with a little cold water to form a thin paste, and then stirring with cold or warm water, which is added by degrees. The floury skim milk powders generally give with cold water a product which does not separate any of the constituents of the milk on standing. Other products require warm water, and even then give a deposit on standing. The fat quickly rises as an oily layer.

Analysis of Dried Milk.—*Water* may be determined by drying a few grams spread in a flat dish at 100° till constant in weight. *Fat* may be determined by the Gottlieb process, as described on p. 263, taking care to warm after adding the water and ammonia. *Proteins* may be determined by the Kjeldahl process, using about 0·2 gram of the sample, and digesting with sulphuric acid and copper sulphate or oxide till clear (see p. 273). *Ash* may be determined by incinerating as in the case of milk (see p. 280). *Lactose* may be determined in the solution as in milk (see p. 276). *Sucrose.* For the detection and estimation of sucrose, see p. 293. *Starch.* This is a possible, though very rare, adulterant of milk powder. It is easily detected by microscopic examination, and the blue colour which it gives with iodine (see pp. 304 to 308). Infants' foods containing starchy matter may be recognised in this way. The determination of starch in infants' foods is described on pp. 311 and 358.

If the ratio of lactose to proteins to ash is as in milk (see p. 283), the sample will be genuine dried milk without, at least, any appreciable addition. Taking the ash in milk as 0·75 per cent. or the proteins as 3·4 per cent., the degree of concentration may easily be calculated, and thence the percentage of fat in the original milk. Milk powder used as infants' food should be made from full cream milk.

The detection and estimation of *preservatives*, which, however, are rarely present, is described in Chapter IX.

THE ANALYSIS OF BUTTER AND MARGARINE.

The composition of butter has already been dealt with at the beginning of this chapter. As a rule, the only essential difference between butter and margarine lies in the composition of the fat. The same methods as are employed for the determination of water, fat, proteins and non-fatty solids in butter may therefore be applied to margarine.

Sampling.—In order to obtain a fair average sample of butter from a large mass, a sampling iron, such as is shown in Fig. 23, is generally used. Two or three cylindrical samples are taken in different directions with the iron, introduced into a wide-mouthed glass-stoppered bottle, melted at a temperature not above 40°, and shaken vigorously until solidified; samples for analysis may then be taken from the bottle as required. The butter should not be kept too long before analysis, as it is liable to alter in composition on the development of rancidity.

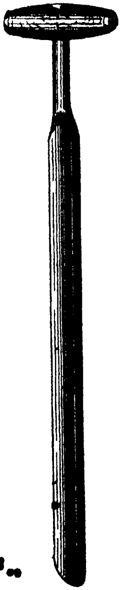

FIG. 23.
Sampling Iron.

Water, Fat, and Non-Fatty Solids.—In Germany the minimum percentage of fat in saleable butter is fixed by law at 80, and the maximum water percentage at 18 for unsalted and 16 for salted butter. In the United Kingdom, no definite minimum fat percentage has been laid down by the law, but, as mentioned above, the maximum water percentage is fixed at 16.

The non-fatty solids include casein, or curd, and small amounts of lactose, lactic acid and inorganic salts derived from the milk or cream, as well as any added salt or boric acid preservative.

About 10 grams of the sample are weighed out in a beaker, or a crucible of porcelain or nickel, about two and a half inches high, with a small stirring rod, and the water is driven off by heating on a sand bath or asbestos wire gauze, taking care not to heat so strongly that loss takes place by spirting or the curd turns brown ; heating is discontinued immediately all the water is seen to have been driven off. The butter should be kept well stirred during the heating, which should not occupy more than about ten minutes. On cooling, the water is determined by the loss in weight. The fat is then melted at the lowest possible temperature, and about 30 c.c. of petroleum ether, volatile below 60°, are added, mixed with the fat and poured off through a tared filter paper or Gooch crucible ; the residue is washed with successive portions of petroleum ether till free from fat. The filtered fat solution is collected in a weighed flask, and the fat is determined after evaporation of the solvent, as described on p. 266. The solid residue, consisting of curd, etc., is estimated by weighing the beaker and the filter after drying in the water oven. Having determined the water and the non-fatty solids, the fat may be estimated by difference instead of directly.

Fig. 24 shows a balance for the determination of water in butter or margarine. 10 grams of the sample

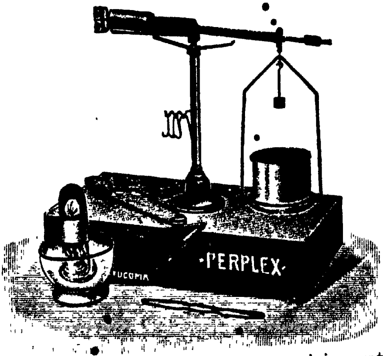

FIG. 24.—"Fucoma" Balance for determining water in Butter or Margarine.

are weighed into the aluminium pot, and the loss in weight after driving off the water is determined by restoring the balance by placing rider weights on the beam as has been explained in connection with the Westphal balance on p. 50. The empty pot may conveniently be balanced by the addition of a little powdered pumice, which also serves to facilitate the driving off of the water without bumping.

Salt and Boric Acid.—These may be extracted from the non-fatty solids by washing with hot water, the contents of the beaker being washed through the filter. The aqueous solution is cooled and made up to a definite volume, and the salt determined in an aliquot portion by titrating with decinormal silver nitrate solution, standardised against pure sodium chloride, using potassium chromate as indicator. About a half of the total solution from 10 grams of the original sample will be required; the solution must be neutral or feebly acid for the titration. Boric acid may also be determined in the solution by the method described on p. 413; but to get an accurate result it is better to operate on a larger amount treated as follows:—

At least 25 grams of the butter or margarine are heated on the water bath for fifteen minutes with an equal number of c.c. of a solution containing 1 gram of sulphuric acid and 50 grams of sodium sulphate per litre, the mixture being shaken occasionally. The aqueous layer is removed by wash bottle tubes and passed through a dry filter. Five c.c. of the filtrate may be used for the salt titration, diluting with 20 c.c. of water, and 20 c.c. for the boric acid titration (see p. 414). In calculating the results, the water contained in the sample must be taken into account.

Proteins.—These may be determined in the non-fatty solids by the Kjeldahl process (see p. 273); it is only necessary to digest with sulphuric acid and copper oxide or sulphate till clear. The proteins may be removed from the beaker or Gooch crucible by solution in a little warm 50 per cent. (by volume) sulphuric acid; if a filter paper has been used, this may be transferred direct to the digestion flask; in this case the blank Kjeldahl test should be carried out with the inclusion of a similar filter paper. The percentage of nitrogen multiplied by 6·38 gives the proteins.

The proteins may also be extracted by warming, say, 20 grams of the sample with an equal weight of a mixture of equal parts by volume of strong sulphuric acid and water, and shaking till the acid and fat layers separate clearly, taking care to dissolve up any particles of curd which may adhere to the side of the vessel. After settling, about 8 c.c. of the acid layer are carefully removed by means of a pipette, the bottom of the pipette is wiped free from fat by means of filter paper, and 10 grams of the liquid is weighed into a Kjeldahl digestion flask, which may conveniently be of 100 or 200 c.c. capacity. Fifteen c.c. of strong sulphuric acid and a little copper oxide are added, and the digestion is carried on till the mixture is clear and colourless or blue (see p. 20). The number of c.c. of decinormal acid equivalent to the ammonia formed (less the blank value) divided by ten, gives the percentage of proteins if the original sample contained from 10 to 14 per cent. of water. The division factor is 9·6 for 14 to 19 per cent. of water. These factors will give the protein percentage with accuracy within the limits of experimental error.

For percentages of salt and proteins, and composition of non-fatty solids, see p. 254.

Examination of the Fat.—The examination of butter fat has already been dealt with in Chapter III., p. 164 *et seq.*, where the problem of distinguishing margarine fats from butter fat, and the detection of the former in the latter, has received attention. As a rule, the only important distinction between butter and margarine lies in the composition of the fat.

Colouring Matter.—Small amounts of colouring matter are added to margarine, and also to butter, in order that it may resemble the grass butter of the summer months, all the year round. The vegetable dyes turmeric and anatto, as well as certain azo-dyes, are employed for this purpose; these are harmless, and added in very small amounts. Various methods by which they may be detected and identified, if desired, are given in Chapter IX.

REFERENCES.

"Methods used in the Examination of Milk and Dairy Products," by C. Barthel, translated by Goodwin. 1910. Macmillan & Co., London.

"Food Inspection and Analysis," by Leach. 1909. Wiley and Sons, New York.

"Milk and its Relation to Public Health," by various authors. 1908. Washington Government Printing Office.

"Foods, their Composition and Analysis," by A. and M. Wynter Blyth. 1909. Chas. Griffin, London.

"Chemie und Physiologie der Milch," by W. Grimmer. 1910. Verlagsbuchhandlung, Paul Parey, Berlin.

"Mykologie der Milch," by Weigmann. 1911. M. Hensius Nachfolger, Leipzig.

"Milchwirtschaft," by W. Kirchner. 1907. Verl. von Paul Parey, Berlin.

"Fatty Foods," by Bolton and Revis. 1913. J. and A. Churchill, London.

"Die Bacteriologie in der Milchwirtschaft," by Orla Jensen. 1913. Gustav Fischer, Jena.

"Dairy Chemistry," by Richmond. 1914. Chas. Griffin & Co., London.

"Laboratory Book of Dairy Analysis," by Richmond. 1913. Chas. Griffin & Co., London.

"The Pasteurisation of Milk from the Practical Viewpoint," by C. H. Kilbourne. 1916. John Wiley, New York.

"The Principles and Practice of Milk Hygiene," by Klein. 1918. Lippincott & Co., Philadelphia and London.

"Milk Hygiene," by Ernst, trans. by Mohler and Eichhorn. 1914. Ballière, Tindall & Cox, London.

"Milk and its Hygienic Relations," by Lane-Claypon. 1916. Longmans Green.

CHAPTER VII

STARCH AND ALLIED PRODUCTS—FLOUR, BARLEY AND MALT, ETC.

INTRODUCTORY

STARCH is one of the most important and characteristic products of the vegetable kingdom, not only on account of the prominent part which it plays in plant physiology, but also on account of its great economic value as a food material for animals and human beings. A considerable portion of the non-nitrogenous reserve food material of plants is, in many cases, stored in the form of starch. The most abundant supplies of this substance are to be found in seeds, tubers, etc., where it remains as such until it can be assimilated by the embryo plant. Starch is one of the most important constituents of the flours used in making bread and many other common articles of food, of rice, which practically forms the staple diet of one-third of the human race, of sago, of tapioca, maize and barley, and of such vegetables as potatoes, beans and peas. As a food starch is used together with the nitrogenous, fatty, saline and other matter with which it is associated in nature.

Starch which has been separated from the other constituents of the grain, usually by washing with water and levigation, is used, as such, for laundry purposes, as a thickening material in calico printing, and for the preparation of adhesive paste.

As regards indirect uses, starch is of importance as a source of alcohol, which results from the action of yeast on the sugar produced from the starch through the hydrolysing action of mineral acids, the enzyme diastase, or certain moulds.[1] In the manufacture of beer, most of the starch contained in the malt is partially hydrolysed by the action of diastase (an enzyme or mixture of enzymes occurring in barley and other seeds) to dextrin and carbohydrates of a similar nature, only part of the starch being converted into sugar which can be fermented to alcohol by the action of yeast. In the manufacture of alcohol and spirits, on the other hand, the starch is practically completely converted into alcohol, as indicated above.

The starches from maize, wheat, rice and the potato are usually employed for the direct applications ; barley is used in the manufacture of beer, whisky and other spirits, while dextrin and starch sugar, for brewing purposes or conversion into alcohol, are generally prepared from starch from the potato or from damaged or inferior rice, maize, etc.

PROPERTIES AND IDENTIFICATION OF STARCHES.

Starch exists in plant cells in the form of well-defined granules, varying in diameter from 0·002 to 0·185 mm. (=2 to 185 μ). When viewed under a microscope of fairly low magnifying power (about 150 to 300 diameters) the granules often show a very characteristic structure, consisting of a hilum, or nucleus, surrounded by a number of concentric rings. In order to show up these peculiarities in structure, which vary with starches from different sources, a small quantity of the powdered starch should be mounted in glycerine diluted with twice its volume of water, and viewed, if possible, under oblique illumination. With practice, it is often possible to determine the source of a starch from the size and shape of the granules and the relation of the concentric rings to the hilum. Further, on examination under a polarising microscope with crossed Nicol prisms, many starches display characteristic arrangements of dark bands, usually giving the appearance of a Maltese cross. If a selenite plate be placed between the lower polarising Nicol prism and the object, colours will be shown with many starches.

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[1] These may also effect the conversion to alcohol.

Starch may readily be identified by the characteristic blue colour which it gives with iodine. This reaction is best carried out by adding to a solution of the starch a dilute solution of iodine in potassium iodide solution, drop by drop ; or, if the starch is being observed under the microscope, a drop of the iodine solution may be placed at the side of the cover slip, so that it will gradually diffuse into the glycerine in which the granules are mounted, when these will be seen to take up a dark blue colour.

The best method for identifying starches is to obtain a collection of genuine flours, starches or natural products containing starches, and when these have been carefully examined, the unknown starch or mixture o starches may be identified by comparison. Help ma also be obtained by consulting the works on microscop mentioned at the end of this chapter. The followin characteristics of some of the common starches may b noted :—

Ovoid Shaped.—Potato and arrowroot starches. In *potato starch,* the hilum is circular and situated in the narrow end of the granule; some of the striations, or rings centring round the hilum, are well marked. In *arrowroot starch,* on the other hand, the hilum is usually elongated, sometimes cleft, and situated in the broad end of the granule, though these features may not be strictly characteristic of all the granules.

Polygonal or Faceted Starches.—Rice, maize and oat starches. *Rice starch* is easily distinguished by the smallness of the granules. The hilum is central and visible under a high power. Some larger rounded granules may be found, and sometimes the granules occur in ellipsoidal aggregates. Rice starch closely resembles *pepper starch,* and the detection of rice flour as an adulterant of pepper requires close examination. It will be found that the granules of pepper starch are not so angular in contour as those of rice starch; they tend to form aggregates. *Cocoa starch* granules also resemble those of rice starch, but are distinctly round in contour; they also tend to form aggregates. Rice starch will more easily be detected in cocoa than in pepper. *Maize starch* granules are much larger and rather more rounded than those of rice starch; the hilum is central and distinct, showing radiating clefts in most of the granules. Maize starch is the only common polygonal starch with granules above 15µ in diameter. The variations are usually from 15 to 30µ. *Oat starch* granules are intermediate in shape and size between those of rice and maize. The hilum is central, generally circular in appearance, and not so distinct as in maize starch. Characteristic ellipsoidal aggregates of granules will be found.

Buckwheat Starch may be described as round or ounded polygonal. The hilum is distinct, and approximately circular and central. Ellipsoidal or circular aggregates are not found, but two or more granules may be united in a rod-like aggregate, the boundary between the granules being indistinct. The granules vary somewhat in size from 2 to 15 μ, mostly 6 to 10 μ, but are generally larger than those of oat starch, which are on an average about 10 μ in diameter.

Round Starches.—Wheat, barley, and rye. In these starches the hilum and striations are not well marked, and will only be seen in some of the granules. The hilum usually appears as single or radiating clefts. Small granules will be observed in addition to the large ones. The larger granules of *wheat starch* are usually from 25 to 30 μ in diameter. *Barley starch* resembles that of wheat, but the larger granules usually measure 15 to 25 μ and rarely as much as 30 μ. Hilum and striations are more rare, and some kidney-shaped granules will be observed which are not found in wheat starch. *Rye starch* also resembles wheat starch, but the larger granules measure from 40 to 50 μ, and the shape is less regular, some kidney-shaped or elongated granules being found.

Bean-shaped Starches.—Bean, pea and lentil starches. *Bean starch* granules are ellipsoidal or kidney-shaped and fairly regular; they are very large and in most cases show very distinct clefts which may be about 60 μ long. The striations are fairly well marked. *Pea starch* granules are more irregular in shape, being ellipsoidal, kidney-shaped, or globular, with protuberances; they are smaller than bean starch granules, being seldom over [illegible]0 μ in diameter, and fewer of the granules show distinct clefts. The concentric rings are well marked. *Lentil starch* granules are rather smaller than those of pea starch. Clefts and striations may be well marked, and some granules with irregular protuberances will be found.

Microscopic examination will be found a useful means of determining the origin of starches or flours, as well as detecting adulteration with foreign starches. The detection of rice starch in pepper or cocoa has already been alluded to, and the detection of rice, potato or leguminous flours in wheat flour will be found a fairly easy problem with some practice. This matter is dealt with further below (see p. 340).

The microscopic examination of vegetable structures is an important feature in the examination of foods and drugs, the recognition of the starches which are characteristic of many products being only a particular case.

Chemically considered, starch is not a definite substance, but consists of a mixture of two carbohydrates, granulose and starch cellulose, or rather, a series of substances which may be looked on as gradations between the two.

Starch cellulose is a body intermediate as regards complexity of structure, stability and other properties, between granulose and ordinary cellulose; from the latter it differs in being convertible into soluble starch by boiling in water or digesting in caustic alkali solution. It is insoluble in cold water and saliva, and gives a yellow colour with iodine. Granulose, on the other hand, is soluble in saliva, and gives the characteristic blue colour with iodine. Ordinary starch is insoluble in cold water; on heating with water, however, the granules begin to swell as the temperature approaches 60°, and on further heating they become disintegrated and mix with the water, giving a solution which gelatinises strongly on cooling, and from which starch may be precipitated on the addition of alcohol. The starch thus obtained is soluble in cold water, and is usually known under the name of soluble starch. The soluble variety is also produced when starch is heated in closed vessels to 100°.

Considered as a member of the carbohydrate group, starch ranks after cellulose in point of complexity, stability towards hydrolysing agents, and solubility; when acted on by ordinary diastase, it is converted, first into dextrin, or rather a series of bodies known collectively as the dextrins, and ultimately into dextrin and the reducing disaccharide maltose. Taka diastase converts it into a mixture of maltose and dextrose, the proportion of the latter increasing with the time of action (see p. 312). Boiling dilute mineral acids convert starch into dextrins, maltose, and finally into *d* glucose, or, as it is commonly called, dextrose. The mechanism of these hydrolyses and the nature of the products formed will be further discussed when the methods for estimating starch are explained.

METHODS FOR ESTIMATING STARCH IN COMMERCIAL STARCHES, FLOURS, POTATOES, BARLEY, MALT, ETC.

(1) *Hydrochloric Acid Method.*—This method, which is prescribed by the American Association of Official Agricultural Chemists (A.O.A.C.), depends on the fact that starch is converted into dextrose on boiling with dilute hydrochloric acid. The dextrose formed may be estimated by means of Fehling's solution, the polarimeter, or by the specific gravity of its solution, and furnishes a measure of the starch present in the sample.

It should be noted that a certain class of gummy bodies known as the pentosans, which are often present in notable proportions in flours, give rise to reducing sugars, *i.e.*, the pentoses (sugars containing five carbon atoms in the molecule), on boiling with acids. The method is therefore only applicable to commercial starches which have been freed from extraneous gummy matter, such as, for example, the starches used for stiffening textiles. Even then the method is not accurate, as the results are some 3 to 5 per cent. too low owing to the destruction of some of the dextrose by the hydrochloric acid.

Three grams of the sample are mixed with about 50 c.c. of cold water, stirring at intervals for about an hour. The insoluble residue is then collected on a filter and washed with water until the filtrate measures 250 c.c. The residue, which has now been freed from soluble carbohydrates, is heated on a water bath for two and a half hours with a mixture of 200 c.c. of water and 20 c.c. of hydrochloric acid of specific gravity 1·125 (*i.e.*, 2·5 per cent. HCl), in a flask fitted with a reflux condenser. The mixture is then cooled, neutralised with sodium carbonate, made up to 250 c.c., and filtered through a dry filter. The dextrose is determined in an aliquot portion of the filtrate by means of Fehling's solution, as described in Chapter VIII., p. 373. The weight of dextrose found, multiplied by 0·9, gives the weight of the starch.

(2) *Polarimetric Methods.*—Lintner's polarimetric method is used in several modifications. The starch is rendered soluble by treatment with mineral acid and estimated polarimetrically after proteins, etc., have been removed from the solution. The method has the advantage of rapidity, and is certainly not inferior to the

preceding one as regards accuracy. It is applicable to all starch containing substances, such as cocoa, potatoes, flours, etc., but suffers from the same disadvantage as the preceding method, *i.e.*, any pentosans present will yield optically active substances which will count as starch. (See also under the Taka diastase method.) It is best to work with fat free material. The modification described here is that of Thorne and Jeffers,[1] which was employed by Baker in the analysis of infants' foods.[2]

Five grams of the finely ground material are triturated in a mortar with 10 to 15 c.c. of water, followed by 15 to 20 c.c. of hydrochloric acid of specific gravity 1·15 added in quantities of 5 c.c. at a time. The starch first forms a viscous mass which soon after becomes thin. After standing for half an hour, the mixture is transferred to a 200 c.c. flask containing 10 c.c. of a 4 per cent. solution of phosphotungstic acid and 20 c.c. of the hydrochloric acid previously used. The mortar is washed out with hydrochloric acid of specific gravity 1·1, and the contents of the flask made up to the mark with acid of this strength. The mixture is shaken and allowed to stand for at least half an hour. The function of the phosphotungstic acid is to precipitate proteins, tannins, etc., and to produce a liquid which will filter clear. The polarimetric reading of the filtrate is taken in a 200 mm. tube in a Schmidt and Haensch polarimeter using white light. Assuming the specific rotatory power of the soluble starch to be +200°, the percentage of starch (*P*) is calculated from the reading (*R*) by the formula :—

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[1] *Analyst*, 1909, 34, 332.

[2] Reports to the Local Government Board on Public Health and Medical Subjects. 1. "On the Use of Proprietary Foods for Infant Feeding," by Dr. F. J. H. Coutts. 2. "On the Analysis and Composition of some Proprietary Foods for Infants," by Mr. Julian L. Baker.

$$P = \frac{R \times 40.}{11{\cdot}6}.$$

This method was found by Baker to give results accurate to within ±0·5 per cent. with mixtures of starch and casein. In order to allow for the optical activity of the sugars present, the following corrections were made on the polarimetric readings before applying the above formula :—

Cane sugar per cent.	×	0·028	added to reading
Dextrose	,,	0·07	deducted from reading
Lactose	,,	0·07	,, ,,

The sugars are determined by methods given in the next chapter.

Another method by which the optical activity of sugars (and dextrins) is compensated for in the polarimetric determination of starch is that proposed by Baumann and Grossfeld.[1] The starch, but not the other carbohydrates, is precipitated by lead tannate, the latter being formed in the starch solution by adding to it basic lead acetate and tannin solutions ; this permits of the necessary control experiment being made. The method is based on Ewers' well-known method for the polarimetric estimation of starch,[2] which is similar to the one described above.

(3) *The Taka Diastase Method.*—This method, due to Davis and Daish,[3] is based on the fact that the enzyme

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[1] Zeitschr. für Untersuch. Nahr. u. Genussmittel, 1917, 33, 97, abs. *J.C.S.* 1917, 112, 223.

[2] Zeit. öffentl. Chem. 1908, 14, 8, and 1915, 21, 232, abs. *Analyst*, 1908, and *J.S.C.I.*, 1916, 35, 432.

[3] *J. Agric. Science*, 1914, 6, 152.

taka diastase converts starch into a mixture of dextrose and maltose, which may be estimated polarimetrically and by cupric reduction. It is free from the defects mentioned in connection with the methods already described. For example, Revis and Burnett[1] demonstrated for the first time by its means that cocoa shell contains no starch, a fact which is obvious from microscopic examination, whereas judging by the results of the hydrochloric acid and polarimetric method, considerable amounts of starch would be indicated owing to the fact that cocoa shell contains substances other than starch which yield reducing and optically active carbohydrates on hydrolysis (see p. 310). The same point is illustrated in a paper by Baker and Hulton on the "Analytical Examination of Acorns and Horse Chestnuts."[2] The taka diastase method is also superior to O'Sullivan's method in which ordinary diastase from malt is used to convert the starch into dextrin and maltose, for it has been shown by Davis and Daish that dextrin is lost by absorption when the proteins, tannins, etc., are precipitated by lead acetate prior to polarimetric examination. For these reasons the method will probably become the standard one for the estimation of starch in plant material.

Preliminary Treatment of Material.—If fat is present, it must first be removed; the dried residue from the fat estimation (see pp. 328 and 87) may be made the starting point, or the original material, *e.g., flour* (10 grams), may be soaked in absolute alcohol, digested for a few hours with about 30 c.c. of ether, filtered, and the residue washed with ether.

[1] *Analyst*, 1915, 40, 420.

[2] *Analyst*, 1917, 42, 351.

Material containing much water, such as *potatoes* (see table, p. 318) or *leaf material*, should be dried Davis and Daish dry *leaf material* to constant weight at 100° or 110° in vacuo over phosphorus pentoxide for twenty-four hours or more.

The next step is to remove sugars and other soluble carbohydrates, which is accomplished by extraction with alcohol and water. The fat free *flour* from the alcohol and ether treatment just described is digested with about 100 c.c. of alcohol of specific gravity 0·90 at 35° to 38° for a few hours, shaking occasionally. The alcoholic solution is passed through the same filter as was used for the alcohol-ether operation, and the residue washed with alcohol of specific gravity 0·90. Extraction with water is necessary to remove gummy material (pentosans; see p. 310) and amylans. The latter are carbohydrates having the same empirical formula as starch, but are soluble in cold water, and give no coloration with iodine. Wheat, for example, contains about 2 per cent. of β amylan. The residue from the last operation is digested with 500 c.c. of water at the ordinary temperature, and then decanted through the filter used previously; the residue is transferred to the filter by means of water, and repeatedly washed with water at 35° to 38°. If much gummy matter is present, prolonged extraction and washing with water is necessary.

Revis and Burnett treat *cocoa* as follows: Five grams of the fat free dry cocoa are weighed into a beaker and thoroughly stirred with 50 c.c. of 10 per cent. (by vol.) alcohol, and filtered by suction on a large Buchner funnel. The cocoa is washed with two further portions of 50 c.c. of 10 per cent. alcohol, and finally with 10 c.c. of 95 per cent. alcohol (by vol.). Care should be taken that the cocoa does not suck dry at any stage, as it will then be more difficult to wash and remove from the filter. Stress is laid on the importance of the alcohol washing for the success of the process. Baker and Hulton (*loc. cit.*) extracted their material with ether and water.

Gelatinisation and Conversion of the Starch.—For this operation, the moist material from the alcohol or water treatment is taken. Davis and Daish give the following directions: The starch is gelatinised and rendered soluble by heating with 200 c.c. of water in a boiling water bath for half an hour, shaking occasionally. The mixture is then cooled to 38°, and 0·01 gram of taka diastase[1] are added together with 2 c.c. of toluene. The function of the latter is to prevent bacterial action which would interfere with the results of the process. The diastase is allowed to act for twenty-four hours at 38°, after which the solution is boiled to stop further action, the diastase being thus destroyed.[2] The clear solution is decanted through a filter into a 500 c.c. flask, and the residue is washed several times with water, the washings being passed through the filter until the volume of the filtrate is about 475 c.c. Five to 25 c.c. of basic lead acetate are added as may be required; a large excess beyond that required to precipitate all the tannins, proteins, etc., should be avoided. After making up to 500 c.c., the mixture is filtered, and 100 c.c. of the filtrate are placed in a 110 c.c. flask (see p. 135), treated with just sufficient sodium carbonate solution to precipitate all the lead, and made up to 110 c.c. Fifty c.c. of the filtrate from the lead carbonate are used for the determination of cupric reduction with Fehling's solution (see p. 373), while the optical activity of another portion is read in a 400 mm. tube. For the determination of maltose and dextrose by these means, see the following chapter, pp. 373 to 379. The amounts of starch corresponding to the dextrose and maltose are found and added together: the weight of dextrose is multiplied by 0·9, and that of maltose is divided by 1·055.

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[1] Obtainable from Messrs. Parke, Davis & Co.

[2] Cf. "The Destruction of Milk Peroxidase," p. 287.

Burnett and Revis follow the above process in all essentials. The moist extracted cocoa (from 5 grams) is transferred to a 250 c.c. flask with boiling water and gelatinised with 125 c.c. of water, while 0·05 gram of diastase rubbed up with a little water, and 2 c.c. of toluene are used in the conversion. The action is stopped by the addition of 10 c.c. of decinormal caustic soda solution; the mixture is then cooled to 15°, 100 c.c. of water and 10 c.c. of Wiley's mercuric nitrate solution (see p. 391) are added, and finally water is added up to the mark, below the toluene. The contents of the flask are mixed and filtered. To 100 c.c. of the filtrate, which should be colourless and bright, half a gram of crystallised disodium hydrogen phosphate is added and dissolved. Ten c.c. of caustic soda solution are then added while the mixture is being agitated; the strength of this solution should be adjusted so that 10 c.c. of it just neutralise 4 c.c. of the acid mercuric nitrate solution; caustic soda should not be left in excess, and it is preferable that the solution should be left slightly acid after adding the alkali. The contents of the flask are mixed and filtered, and 50 c.c. of the filtrate are used for the determination of the cupric reducing power, the optical activity being determined on another portion (see p. 378). Revis and Burnett state that taka diastase keeps well in the dark, and recommend that a blank experiment should be made with it in order that any cupric reducing power or optical activity which it may be possessed of may be allowed for. They suggest an allowance of 3 c.c. for the volume of the precipitate.

Baker and Hulton (*loc. cit.*) found it necessary to repeat the gelatinisation and treatment with taka diastase before all the starch in the material examined by them was converted.

FLOUR.

INTRODUCTORY.

The most important of the starch-containing food materials are derived from the grain of the cereals, such as wheat, rye, maize, barley, etc., after removal of the chaff (paleæ and glumes) by threshing. The coarsely ground grain is known as meal, the finely ground grain as flour. Special attention is paid to the examination of wheaten flour, as this is the most important of the flours consumed in this country, as well as being the dearest, and therefore the most likely to be adulterated with other flours.

The following table contains results published by the United States Department of Agriculture, showing the average composition (percentage) of the more important cereals; a typical analysis of potato is appended.

The products obtained from the grain of wheat on milling may be divided into the flour proper, by which is understood the contents of the parenchymatous cells of the endosperm, and the offal, which includes the bran, or husk, the germ, *i.e.*, the embryo plant, and fluffy or fibrous matter derived from the cell walls of the parenchyatous endosperm; "sharps," or "middlings," consist f finely divided bran with particles of germ. The commercial valuation of flours varies according to the amount

Description of Grain.	Moisture.	Nitrogen × 6·25.	Crude Fibre.	Ash.	Carbohydrates other than Crude Fibre.	Ether Extract (chiefly fat).	Weight of 100 Kernels in Grams.
Typical unhulled barley . .	10·85	11·0	3·85	2·5	69·55	2·25	—
Typical American maize . .	10·75	10·0	1·75	1·5	71·75	4·25	38·0
Typical rye .	10·5	12·25	2·1	1·9	71·75	1·5	2·5
Typical unhulled oats . .	10·0	12·0	12·0	3·4	58·0	4·5	3·0
Typical rice, unhulled .	10·5	7·5	9·0	4·0	67·4	1·6	3·0
Typical rice, polished . .	12·4	7·5	0·4	0·5	78·8	0·4	2·2
Typical wheat .	3·85	12·25	2·4	1·75	71·25	1·75	3·85
Potato . .	74·7	2·0	1·4	1·0	20·7	0·2	—

of offal which they contain, the highest grade being the so-called "patent flour" which, owing to its freedom from offal, is the whitest in colour and most homogeneous product; it is appreciated by bakers on account of the amount of water which it will absorb and the appearance of the loaf which it produces, though as regards nutritive value it is not necessarily superior to the lower grades. "Wholemeal," or "Graham," flour is the product of grinding of the entire wheat grain, including the husk and germ; it should have the same composition as the wheat grain itself; containing the whole of the offal, it is considerably darker in colour than patent flour, and not being subjected to any sifting process, it contains branny particles which are distinctly visible. The so-called "entire wheat flour," or fine meal, is the product obtained by removing a portion of the bran and finely grinding the rest of the grain ; the texture is somewhat coarser than that of ordinary or patent flour, the colour and general appearance varying somewhat, according to the amount of offal removed and the nature of the wheat from which it is milled. "Entire" flour usually contains a portion of the germ. "Standard," or 80 per cent. flour, may be classed as an "entire" flour, representing 80 per cent. of the wheat grain, and containing the whole of the germ. The Manufacture of Flour and Bread Order (No. 2) of 1917 made it compulsory to extract from the wheat not less than 81 per cent. of flour on all millers in the United Kingdom. In April, 1918, it was laid down that in the manufacture of Canadian "Government Standard Flour" 196 lbs. of flour must be milled from 258 lbs. of spring wheat instead of from 270 lbs. as previously. These measures were due to the scarcity of wheat during the latter period of the war. Regarding the addition of flour other than wheat flour, see p. 340.

H. E. Cox, in a paper on the Composition of "Sharps" and Bran" and the effect thereon of the Food Controller's Orders,[1] gives the following example of the results of grinding into various grades of flour, sharps (or middlings), and bran in pre-war days : *Flour,* finest, 42 per cent. ; seconds, 18 per cent. ; biscuit, 8 per cent. ; tailings, 2 per cent. *Sharps*—middlings, 10 per cent. ; coarse sharps, 8 per cent. *Bran*—fine, 4 per cent. ; long, 8 per cent.

"Households grade" is the commercially lower grade of flour which is produced besides the patent grade in the modern process of roller milling ; it is darkish in colour and contains a small amount of fine branny articles; from the baker's point of view, it is inferior o patent flour in bread making. "Baker's grade" and 'clear grade" are terms applied to similar grades in America. "Straight run," or "straight grade," may be regarded as a mixture of the patent and household grades.

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[1] *Analyst,* 1918, 43, 53.

Special flours may be prepared from any of the above grades, usually with a view to improving the nutritive value. They may contain finely powdered bran, lentil flour or banana meal. Germ flours contain added germ (usually cooked).

Much of the above is an abridged version of Dr. Hamill's classification of the various flours on the market, included in his report to the Local Government Board on the nutritive value of bread made from different varieties of wheat flour.[1] As is pointed out by Dr. Hamill, it is very difficult to set up analytical standards for defining any particular grade of flour, owing to the difference in composition of wheats from different sources. Comparisons of the composition of different grades of flour can only be of value when these are milled from the same wheat.

The following table, compiled by the United States Department of Agriculture, shows the variations in the composition of wheats from different sources:—

	Per cent.
Water	7 to 14
Proteins	8 „ 17
Dry gluten	2 „ 14
Carbohydrates	65 „ 76
Ether extract (chiefly fat)	0·28 „ 2·5
Ash	1·4 „ 2·3

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[1] Reports to the Local Government Board on Public Health and Medical Subjects, New Series, No. 55, Food Report, No. 14

A number of analyses of various flours and other milling products obtained from different wheat mixtures are given in the above report. From these and from the figures given in the next table, it will be seen that the presence of bran in the flour tends to raise the content of mineral matter and crude fibre. The presence of germ tends to raise the content of fat and protein, but usually these increases are comparatively insignificant. "Germ flours" may, however, contain as much as 4 to 5 per cent. of additional protein due to added germ.

The following table from Wynter Blyth's "Foods, their Composition and Analysis," shows the composition of various flours of different grades, the original wheat from which these are milled, and of the bran. The superiority of grade and freedom from bran decrease from Nos. 0 to 9:—

Description of Flour of Varying Grades as obtained from Steel Roller Mills and Bran.	Per Cent. Yield on Original Wheat.	Water.	Insoluble Nitrogenous Matter.	Soluble Nitrogenous Matter.[1]	Fat.	Carbohydrates other than Crude Fibre.	Crude Fibre.	Ash.
Original wheat . .	—	13·37	10·69	2·93	1·98	80·41	1·90	2·09
Flour No. 0 . .	6·0	12·56	8·38	3·06	0·83	87·26	trace.	0·47
" " 2 . .	6·0	12·48	8·87	2·95	0·97	86·69	"	0·52
" " 6 . .	4·0	12·39	9·38	3·00	1·17	85·87	0·02	0·56
" " 8 . .	5 to 6	11·72 to 12·41	10·06 to 14·34	2·72 to 3·22	1·30 to 3·51	75·90 to 84·55	0·06 to 1·03	0·81 to 2·29
" " 9 . .	3·0	10·64	15·02	2·55	4·02	74·20	1·55	2·66
Fine bran . .	16·0	11·35	13·50	3·06	4·54	63·64	8·7[illegible]	6·55
Coarse bran . .	2·0	12·37	13·44	3·17	3·46	62·13	9·79	8·01

[1] Soluble nitrogen, estimated by Weinwurm, according to the method described below.

The crude fibre is the cellulose vegetable tissue which is insoluble in dilute boiling acid and alkali; it cannot be looked on as a definite constituent, the amount found varying somewhat according to the method of estimation.

Many of the published figures do not include the percentages of starch and pentosans. From what has been said in dealing with the estimation of starch, it will be gathered that results obtained by the older methods are not very accurate. The pentosans which may be regarded as standing in a somewhat similar relationship to the pentoses as the starches and celluloses to the hexoses, are mainly present in the sharps or bran portion of the grain, and the estimation of these bodies furnishes an indication of the degree of bolting, *i.e.* sieving, of the flour (see p. 334).

On examination of the ash, or mineral matter, the principal bases which will be found are potash, lime and magnesia, and the principal acid, phosphoric acid. The nitrogenous constituents of wheat flour may be divided into the gluten and the soluble nitrogen compounds; they are of importance, not only on account of their nutritive value, but also on account of the texture which they impart to the bread. Gluten consists mainly of two proteins, gliadin and glutenin. The former is a soft, sticky substance which can be pulled out into threads; it may be separated from the other constituents of flour by extraction with 70 per cent. alcohol, in which it is soluble, and by precipitation from this solution by the addition of an aqueous solution of sodium chloride. Though soluble in pure water, gliadin is not dissolved when flour is treated with water, owing to the presence of mineral salts. Glutenin is distinguished by its solubility in very dilute alkali solution; it is of a firmer consistency than gliadin; the latter imparts tenacity to the gluten, and on this account bakers usually prefer a flour containing a high percentage of gliadin. The proteins other than gluten include a globulin, an albumin and a proteose; these are probably of greater nutritive value than the gluten.

THE EXAMINATION AND ANALYSIS OF FLOUR.

As has been previously pointed out, the commercial value of wheat flour depends on its colour, texture, and the amount and nature of the gluten present. The highest grade flours should be practically white, showing only the faintest tinge of yellow; they should possess a sweet smell and be free from branny particles and acidity. By the tests and analytical methods now to be described, the quality of a flour may be gauged to a certain extent; a great deal, however, depends on the appearance of the flour and the loaf which it will make. The detection of added foreign matter and foreign flours will also be dealt with.

Gluten Test.—In this test, the gluten is separated from the other constituents of the flour by mechanical means, examined and estimated. About 30 grams of flour are made into a stiff dough with about 12 to 15 c.c. of water, and allowed to stand for an hour. The mass is then carefully kneaded in a stream of running water, over muslin, until all the starch has been removed. The fresh gluten thus obtained should only have a faint yellow tinge and should be of such a consistency that it can be pulled out into threads; the gluten from English wheat is usually very soft and sticky, having less elasticity than gluten from other wheats. A dark and viscous gluten with a soapy feel indicates the presence of rye flour; most of the other common flours also give more or less coloured glutens, thus, that from barley is non-viscous and dirty reddish brown; from oats, dark yellow; from maize, yellowish and non-elastic; from leguminous flours, such as those of the bean or pea, greyish red to green. The presence of rice or maize flours may give a granular feel to the gluten (see footnote, p. 342). A grey or reddish gluten may also be obtained from inferior wheat flour. After washing, the gluten is left under water for an hour, after which the excess of water is removed as completely as possible by pressing with the hands, and the moist gluten weighed. It is then dried at 100° till constant in weight, which may take 24 hours or more. A good wheat flour will contain from 20 to 40 per cent. of moist gluten and about 10 to 18 per cent. of gluten dried at 100°. When heated to 150°, good gluten swells and assumes the appearance of bread.

The above test, although useful for valuating flour, cannot, of course, be looked on as an accurate analytical process; the gluten obtained will contain small percentages of non-gluten proteins, mineral matter, fat, starch, fibre, etc.

Total Proteins.—The total nitrogen is determined in about 2 to 3 grams of the original flour by the Kjeldahl method, as described in Chapter I., p. 20. The nitrogen present as nitrates, which exist in small quantity in wheat, will not be converted into ammonia and estimated unless the special method is used (see p. 27). The average nitrogen content of wheat proteins being 17·6 per cent., the nitrogen found should be multiplied by 5·68, in order to give the total proteins. For many other cereal proteins, the factor 6·25 gives a more accurate result. Reference to the last table will show that there is a tendency for the protein content to increase in passing from the high-grade flours, through the lower-grade flours, to bran. In this connection, however, it must be remembered that the protein content of wheat from different sources varies within fairly wide limits (see the previous tables). As was pointed out above, the inclusion of the germ in the flour will not materially raise the protein content, though if much extra germ be added, as in the case of the so-called germ flours, the protein content may be raised by as much as 4 to 5 per cent. above the normal.

According to the United States standard of purity, wheat flour must contain not less than 1·25 per cent. of nitrogen.

Gliadin.—This may be estimated by extracting the flour for 2 hours with 70 per cent. alcohol, filtering, and determining the nitrogen in an aliquot portion of the filtrate. For conversion of the nitrogen to gliadin the factor 5·68 should be used.

In a good flour the gliadin should constitute about 60 per cent. or more of the total gluten.

Soluble Nitrogenous Matter.—This is estimated by Weinwurm as follows: 10 grams of the material are treated with 200 c.c. of hot water and 0·5 c.c of acetic acid, and the whole is warmed on a water bath for 20 minutes. The solution is cooled, made up to 500 c.c., and filtered; the soluble nitrogen is then estimated in 50 c.c. of the filtrate, which should be evaporated nearly to dryness before adding the sulphuric acid for the Kjeldahl estimation. Our knowledge regarding the relative digestibility of the various proteins of the cereals is somewhat limited, though it may perhaps be presumed that the soluble nitrogenous matter as estimated by the above method has greater nutritive value than the proteins of the gluten. Several other methods have been devised for estimating the "digestible nitrogenous matter."

Water.—One to 3 grams of the flour are weighed out between watch glasses, and dried in a steam oven till no further loss in weight takes place.

Shutt and Moloney[1] find that on drying in an air oven at 100°, constant weight is not reached in less than 48 hours, and that higher percentages owing to completer drying are obtained on heating for 5 hours at 100° in a vacuum oven in which a steady vacuum of 29·5 inches is maintained. In these experiments about 2 grams of the flour were weighed in small aluminium dishes with tightly fitting lids.

In the United States and Canada, the maximum limit for water in flour is fixed at 13 per cent. An unduly large proportion of water impairs the keeping qualities of the flour, besides, of course, lessening its nutritive value.

Ash.—About 5 grams of the flour are burnt in a crucible, in a muffle furnace, and the residual ash weighed. An alternative method which is sometimes used is to mix the flour with powdered ammonium nitrate, heating the mixture carefully and withdrawing the flame directly fusion commences. In this way, the flour may be burnt up quickly, without the use of the muffle furnace. A corresponding quantity of ammonium nitrate should be heated separately, and the residue which it leaves, if any, determined and deducted from the ash found in the actual estimation.

The amount of ash or mineral matter present in the patent and other higher grades of flour is usually under 0·5 per cent.; households and "standard," or 80 per cent. flour, generally contain between 0·5 and 0·9 per cent. of mineral matter, while wholemeal may contain between 1·5 and 2 per cent., or even more. From a study of the table on p. 321, it will be obvious that the greater the proportion of bran or offal left in the flour, the more mineral matter will the latter contain. According to the United States standard of purity, wheat flour should not yield more than 1·0 per cent. of ash. If the amount of ash found seems abnormally high in relation to the quality of the flour, it is possible that mineral matter has been added with a view to improving its appearance. Acid potassium, magnesium or calcium phosphates may sometimes be added as "improvers" in the proportion of about 0·5 per cent. of the flour. If such additions are suspected, the ash should be preserved for further examination. Alum and copper sulphate may also be used as improvers, but the quantities added will be too small to be detected by any increase in the proportion of the ash. Self-raising flours usually contain added sodium bicarbonate and acid calcium phosphate, these constituents sometimes being added in the proportion of about 3 per cent. of the flour. The detection of added mineral matter will be dealt with later.

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[1] Trans. Roy. Soc. Canada, 1917, abs. *Analyst*, 1918, 293.

Starch.—For this estimation the taka diastase method is to be recommended (see p. 312).

If determined by this method, the percentage of starch will probably afford a useful indication as to the degree of bolting of the flour, as sharps and bran contain considerably less starch than the flour proper. Cox[1] estimates the percentage of starch in "pre-war sharps" at about 25, and in the endosperm of wheat at about 70.

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[1] See Footnote, p. 319.

As the result of the Food Controller's orders, he estimates that the percentage fell to a minimum of 18 in sharps. It should, however, be noted that these figures were based on results obtained by Ewer's polarimetric method, which would certainly be too high.

Acidity.—Twenty grams of the flour are shaken with 200 c.c. of water at intervals, for 2 hours, the mixture s filtered, and 50 c.c. of the filtrate titrated with decinormal sodium hydroxide solution, using phenol phthalein as indicator.

According to Wynter Blyth, normal wheat should show an acidity corresponding to not more than 0·16 to 0·25 per cent. of lactic acid. The test is useful for detecting the presence of unsound wheat in flour.

Fat (Ether Extract).—This is determined by extracting about 3 grams of well-dried flour with ether (see p. 115). The extracted material should be preserved for the estimation of crude fibre. When the extraction is complete, the ethereal solution is evaporated in a tared flask, the residue dried at 105°, and weighed.

Although, generally speaking, it may be said that the greater the proportion of bran in the flour the higher the fat percentage (see the table on p. 321), this can hardly be taken as an index of the quality of the flour, chiefly owing to the variations in the fat content of flours from different sources.

Crude Fibre.—The following method is generally used in England: About 3 grams of the ground sample (the residue from the fat extraction may conveniently be used) are boiled for half an hour with 125 c.c. of a 2 per cent. solution of sulphuric acid, loss of water due to evaporation being continually made up. The mixture is diluted with a few hundred c.c. of water and allowed to stand for some time, after which the bulk of the liquid is filtered off, any matter transferred to the filter being washed back to the original vessel. The residue is then boiled with 125 c.c. of 2 per cent. caustic potash solution, diluted as before, and filtered by suction on a Buchner funnel through two pieces of hardened filter paper which have previously been counterpoised against each other. The bottom piece is to be used as a counterpoise in the weighing of the fibre. The whole of the fibrous matter is washed on to the filter and washed with boiling water till the washings are no longer alkaline, then with a little dilute acid followed by water until the washings are no longer acid. It is then washed with several portions of methylated spirit, and, if fat is present, with several portions of ether. The fibre is finally dried to constant weight in the water oven.

The following method is recommended by the American Association of Official Agricultural Chemists for the estimation of crude fibre in grain, flour, and bye-product cattle foods, such as oil cake, etc.: Two grams of the material are extracted with ether, or the extracted material from the fat determination may be used. The fat free material is placed in a 500 c.c. flask, and 200 c.c. of boiling 1·25 per cent. sulphuric acid are added; the flask is connected with a reflux condenser by means of a rubber stopper; boiling is commenced at once and continued for 30 minutes. A current of air passing through the liquid may serve to reduce frothing. The mixture is filtered, and the residue washed with boiling water until the washings are no longer acid; it is then rinsed back into the same flask with 200 c.c. of a boiling 1·25 per cent. solution of sodium hydroxide, free or nearly so, from carbonate; boiling is commenced at once, and continued for 30 minutes, in the same manner as directed above for the treatment with acid. The insoluble residue is filtered off on a Gooch crucible and washed with boiling water until the washings are neutral, dried at 110° and weighed ; it is then incinerated completely ; the loss in weight thus occasioned represents crude fibre.

The filter used for the first filtration may be of linen, glass wool, asbestos or any convenient form giving clear and reasonably rapid filtration. The strength of the acid and alkali solutions employed should be verified by titration, and not merely by the specific gravity.

According to the United States standard of purity, wheat flour should not contain more than 0·5 per cent. of crude fibre. As will be seen from the table on p. 321, the crude fibre in the highest grades of wheat flour only amounts to traces, and increases with the proportion of bran present. Provided that a perfectly uniform method of estimation is adopted, the percentage of crude fibre is, perhaps, the most reliable index of the amount of branny matter in the flour. The determination is also of importance in the analysis of feeding stuffs for cattle.

Pentosans.—The estimation of these bodies depends on their hydrolysis to pentoses by hydrochloric acid ; the pentoses are converted into furfuraldehyde, or furfural, by the action of the acid ; furfural is volatile, and may be estimated in the distillate by several methods, the best of which are (*a*) by the reducing action on Fehling's solution and (*b*) as the compound formed with phloroglucinol.

With respect to the significance of the estimation, see p. 310, and the notes at the end of the description of the process.

Phloroglucinol Method.—The following procedure is adopted by the American Association of Official Agricultural Chemists: The phloroglucinol which is to be used is tested for diresorcinol by dissolving a small quantity in a few drops of acetic anhydride, heating almost to boiling, and adding a few drops of concentrated sulphuric acid. A violet colour indicates the presence of diresorcinol; if more than a faint coloration is obtained, the phloroglucinol may be purified as follows: About 300 c.c. of hydrochloric acid of specific gravity 1·06 (12 per cent.) are heated in a beaker and 11 grams of the phloroglucinol are added little by little, stirring constantly until it has almost completely been dissolved; insoluble impurities may be disregarded. The hot liquid is poured into a sufficient quantity of cold hydrochloric acid of the same strength, to make the volume up to 1500 c.c., and the whole is allowed to stand for several days for the diresorcinol to crystallise out. The solution is filtered immediately before use; it may be yellow, but this is no detriment.

A quantity of the material which will yield not more than 0·3 gram of phloroglucide is placed in a distilling flask with 100 c.c. of hydrochloric acid of specific gravity 1·060, and a few pieces of ignited pumice. The flask is heated on a wire gauze so as to distil 30 c.c. in about 10 minutes, the distillate passing through a small filter paper as it leaves the condenser. Each 30 c.c. distilled is replaced by 30 c.c. of fresh acid added from a tap funnel in such a way as to wash down the material adhering to the sides of the flask. When 360 c.c. of distillate have been collected, the distillation is stopped, and a solution of phloroglucinol is gradually added to the distillate, stirring well; the amount of phloroglucinol added should be about double the amount of the furfural expected. A darkening through yellow and green will occur, the precipitate which forms finally becoming nearly black. The liquid is made up to 400 c.c. with hydrochloric acid if specific gravity 1·060, and allowed to stand overnight.

The amorphous black phloroglucide is filtered off on a Gooch crucible, washed with 150 c.c. of water so that it does not suck dry before the end of the washing, dried for 4 hours in the water oven, and cooled and weighed in a weighing bottle. Kröber, who has made a study of this method, gives the following data for calculating the amount of furfural, pentoses and pentosans :—

To the weight of the precipitate is added 0·0052 gram to compensate for the loss in washing, in each case. If the weight of phloroglucide, *w*, is under 0·03 gram, the factor for conversion to furfural is 0·5170, to pentoses, 1·0170, and to pentosans, 0·8949. If *w* is from 0·03 to 0·300 gram, the factors are 0·5185, 1·0075 and 0·8866, respectively. If *w* is over 0·300 gram, the factors are 0·5180, 1·0026 and 0·8824 respectively.

Fehling's Solution Method.—Baker and Hulton[1] recommend the following method : One to 2 grams of the material (or 20 to 30 c.c. of the solution) are submitted to the distillation with hydrochloric acid as described above, and a few c.c. of the distillate are tested from time to time by the following method to ascertain when all the furfural has distilled over. Ten c.c. of pure aniline are dissolved in 10 c.c. of glacial acetic acid and 80 c.c. of alcohol (80 per cent.). This reagent produces a violet coloration with furfural ; care must be taken to have so much sodium acetate present that the only free acid is acetic acid, as the coloration is not obtained in the presence of hydrochloric acid; towards the end of the distillation, 5 minutes must be allowed for the coloration to develop. When the distillation of the furfural is complete, which is usually the case when 200 to 300 c.c. have passed over, the acidity of 10 c.c. of the mixed distillate is determined by titration with half normal caustic soda solution. An aliquot portion of the distillate is carefully neutralised with caustic soda solution so as to avoid local overheating which may occasion loss of the volatile furfural. Twenty c.c. of Fehling's solution (see p. 374) are used in a total volume of 100 c.c., *i.e.*, 50 to 70 c.c. of the distillate are neutralised, made up to 80 c.c. and treated with 20 c.c. of Fehling's solution. Baker and Hulton, however, recommend the use of larger amounts in the same proportions in order that larger amounts of copper oxide may be weighed. The solutions are mixed in a conical flask, which is connected with a reflux condenser, and heated for 35 minutes. Heating in boiling water is recommended in preference to heating to boiling over a flame, for it is shown that the blank value obtained with Fehling's solution and sodium chloride alone is greatly reduced by this method without affecting the furfural to copper factor. This value is determined by heating together under the same conditions as in the actual determination, the same amount of sodium chloride as that calculated to be present in the neutralised distillate with Fehling's solution under the same conditions of dilution. The amount of copper oxide thus found is deducted from that found in the actual determination. The precipitate of cuprous oxide is treated as described on p. 277. The factor furfural :

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[1] *Analyst*, 1916, 294.

copper was found to be 0·3 to 0·32, *i.e.*, 3 mg. of CuO corresponds approximately to 1 mg. of furfural.

Koning and Mooij[1] have published the following data showing how the percentage of pentosans varies inversely with the proportion of bolted flour :—

(Average figures are given in this table.)

	Water per cent.	Pentosans on Dry Material per cent.	Pentosans per cent. Calculated.
Unbolted flour . .	14·2	6·25	
Bolted flour	12·1	2·48	
Bread from unbolted flour	50·22	6·23	
Bread with 25 per cent. of unbolted flour .	49·42	5·53	5·30
Bread with 35 per cent. of unbolted flour .	49·33	5·03	4·88

Colorimetric Method.—Spica[2] hydrolyses 10 grams of the fat free flour (see p. 328) and distils the insoluble residue with hydrochloric acid till the whole of the furfural has passed over. The distillate is made up with 95 per cent. alcohol to make a solution containing 50 per cent. of alcohol, and this solution is treated with a solution of aniline acetate in acetic acid (see above). The coloration produced is compared with that obtained from a solution of 0·05 gram of furfural in 1000 c.c. of 50 per cent. alcohol under similar conditions. Practical tests, with flour bolted to yield 60, 80 and 100 per cent., gave solutions producing colorations corresponding to 0·025, 0·152 and 0·225 gram of furfural respectively.

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[1] Chem. Weekblad, 1914, 11, 1064, abs. *Analyst*, 1915, 234.

[2] Ann. Chim. Applic., 1916, 6, 26, abs. *Analyst*, 1916, 305.

Several methods for the determination of pentosans have been discussed, as this matter, like the estimation of true starch, is a fairly recent development and one which will probably repay further study.

THE DETECTION AND ESTIMATION OF FOREIGN MATTER AND ADULTERANTS IN WHEAT FLOUR.

Under this heading the detection of added mineral matter, foreign flours or starches, and flour damaged by fungi, such as ergot, are considered. Added mineral matter cannot, as a rule, be detected with any certainty by an increase in the ash content of the flour, owing to the variations in ash content of flours of differing fineness and origin; for this purpose, the special methods described below must be adopted. Addition of foreign flours, etc., may sometimes be detected by the use of the microscope, though for this task considerable experience is often necessary. In some cases, however, chemical methods are available.

Alum.—Alum is sometimes added to bad or slightly damaged flour by the miller or the baker in order to improve its appearance; although it is only added in very small quantities, which can hardly be injurious to health, its use is prohibited by law in England. Two methods are available for the detection of alum in flour, *i.e.*, the logwood method and the chloroform method; the latter method also allows of the detection of mineral additions other than alum.

Logwood Method.—Tincture of logwood, which should always be freshly made, is prepared as follows: Half a gram of fine logwood chips, preferably cut fresh from the log, is macerated for 10 hours in 15 c.c. of alcohol; 10 c.c. of the solution are poured off and mixed with 150 c.c. of water and 10 c.c. of a saturated solution of ammonium carbonate. The test should be carried out immediately after the addition of the ammonium carbonate; 50 grams of the flour are made into a thin paste with water, a few drops of the logwood solution are added, and the mixture allowed to stand for several hours. If alum is present a lavender blue lake will be produced. The colour should persist when the sample is placed in an oven at 100° for two hours. If only 1 part of alum in 1,000 be present, the flour becomes pink instead of lavender. In the case of a negative result being obtained by this test, the absence of alum may be inferred. If a positive result is obtained, it is best to confirm it by the chloroform method in order to make sure that the coloration is not due to adventitious clayey matter from the millstones; in the modern process of milling, however, in which steel rollers are used, the latter source of contamination is avoided. Moreover, the logwood test is only obtained in the presence of aluminium compounds soluble in water.

Chloroform Method.—This method also allows of the detection of mineral matter other than alum; as mentioned above, alum and copper sulphate are only added in very small quantities, their function being to increase the whiteness of the flour; their use is forbidden by law, and it is but rarely that they will be found in commercial samples of flour. Substances such as acid potassium, magnesium or calcium phosphates, notably the latter, are used in larger quantities, their function likewise being to make lower grades of flour appear equal to the highest grades; they are said to "make the flour bake whiter, while the bread is improved in boldness, texture, crust, etc." Dr. Hamill, in his report to the Local Government Board,[1] states that he has seen acid calcium phosphate added to, and intimately mixed with, flour in the proportion of about 0·45 per cent. of the finished flour. Curtel[2] describes a preparation sold under the name "Blanc Flour," consisting essentially of acid calcium phosphate, which the makers recommend should be added in the proportion of 1 per cent. of the flour. Considerable quantities of calcium sulphate may be introduced if inferior grades of calcium phosphate are used, especially in the case of self-raising flours, which are now usually prepared by adding calcium acid phosphate and sodium bicarbonate to the flour. As mentioned above, the amount of mineral matter added in such cases is usually large enough to be detected by an examination of the ash of the flour. In a report to the Local Government Board it is recommended that bakers and millers should insist on a calcium phosphate containing under 10 per cent. of calcium sulphate. If a conspicuous excess of the latter be found, prosecution under the Sale of Foods and Drugs Act of 1875 might be considered.

The detection of added mineral matter by the chloroform method may be carried out as follows: 200 grams of flour are shaken in a separating funnel with sufficient chloroform to give a perfectly liquid mixture, and allowed to stand overnight. The chloroform is of sufficient density to allow the organic constituents of the flour to float, while the mineral matter will sink to the bottom, without being dissolved. The solid matter which has collected at the bottom is carefully removed through the stopcock, shaken a second time with a little more chloroform, and when it has subsided again, transferred to a watch glass so that the chloroform may evaporate. The residue is treated with a small quantity of water, the solution is separated from the insoluble portion and allowed to evaporate, when the alum will separate, if present, in the form of octahedral crystals, which may be identified by examination under a low-power microscope. The crystals may be dissolved in a little water, and identified as alum by the usual tests. The residue insoluble in water may be examined under the microscope, and subjected to a qualitative analysis for metals and acid radicles; small amounts of copper salts, either in the soluble or insoluble portion, may be detected by their delicate reaction with potassium ferrocyanide.

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[1] *Reports to the Local Government Board on Public Health and Medical Subjects, New Series, No. 49, Food Report No. 12, 1911.* "On the Bleaching of Flour and the Addition of so called 'Improvers' to Flour," by Dr. J. M. Hamill, and "On the Chemical Changes produced in Flour by Bleaching," by Dr. G. W. Monier Williams.

[2] *Annales des Falsifications*, 1910, p. 302.

For the detection of calcium phosphate in flour, Curtel (*loc. cit.*) recommends the following method, which, in principle, is practically identical with the foregoing: Five grams of flour are shaken with 40 to 50 c.c. of carbon tetrachloride, the mixture is centrifuged, and the flour and the carbon tetrachloride are separated from the sediment; the latter is dissolved in a little nitric acid, and tested with a small quantity of ammonium molybdate solution; if the flour contained added calcium phosphate, a copious precipitate will be obtained; the small amount of sediment obtained from unadulterated flours, representing natural impurities, will produce no precipitate. The use of the centrifuge, both in this and the foregoing method, greatly facilitates the separation of the mineral sediment from the flour. The advantage of the two methods just described lies in the fact that practically only the mechanically admixed matter is separated; moreover, by the use of such liquids as chloroform or carbon tetrachloride, interaction between the added mineral matter and the natural constituents of the wheat is avoided, such as, for example, would occur between alum and the wheat phosphates in presence of water.

Detection of Flour Damaged by Moulds.—After bad seasons, flour is liable to contain matter which has been damaged by moulds, especially ergot. For the detection of starch which has been affected by moulds, a minute quantity of the flour may be mounted in a drop of glycerol, and examined under a low-power microscope; a drop of aniline violet solution is then allowed to diffuse into the mounting medium, under the cover glass; granules which have been damaged by mould of any kind will take up the colour intensely.

Chemical Method for Detecting Ergot.—The following method is given by Leffmann and Beam: Ten grams of flour are macerated for about 30 minutes with a mixture of 20 c.c. of ether and 10 drops of dilute sulphuric acid (1 to 5 parts water); the mixture is filtered and the residue is washed with ether until the filtrate measures 15 c c. This filtrate is shaken up with 5 drops of a saturated solution of sodium bicarbonate. The green chlorophyll remains in the ether; the bicarbonate solution will remain clear if the flour is derived from sound grain, but becomes deep violet if ergot is present.

The sale of ergotised flour as good flour would constitute an offence under the Sale of Foods and Drugs Act.

The Detection of Foreign Flours in Wheat Flour.—According to Wynter Blyth the following have been fraudulently added to flour: rye, rice and barley meals, flours from various leguminosae, such as the bean or pea, linseed meal, buckwheat, potato and some other starches. Such additions are stated to be rare in this country, the foreign flours most likely to be met with being those of rice and the potato; admixtures of this nature are, naturally, most frequently to be met with in practice in times of scarcity of wheat. Thus, in 1917, the Ministry of Food laid down 10 per cent. as the minimum and 25 per cent. as the maximum of flours from other cereals to be mixed with wheat flour. An order in 1918 permitted the use in the manufacture of bread of such quantities of potatoes as the maker might think fit.

General Tests.—Vogel extracts the suspected flour with 70 per cent. alcohol, to which 5 per cent. of hydrochloric acid has been added; if the flour is derived from pure wheat or rye, the alcohol remains colourless, but takes up a yellow colour if either barley or oats be present, orange yellow in presence of pea-flour, and purple red or blood red in presence of mildewed or ergotised wheat, respectively.

The conclusions which may be drawn from the appearance of the gluten are given under the gluten test.

The microscopic examination and identification of starches has been dealt with on pp. 304 to 308. The wash water from the gluten test may conveniently be used for microscopic examination for starches.

Potato Starch.—Donné's test for the detection of potato starch in wheat flour is carried out as follows: The flour is examined in a thin layer, mounted in water, in the usual way, under the microscope; then, while the starch is still under observation, a weak solution of potassium hydroxide is allowed to diffuse under the cover glass. Under the influence of the alkali, potato starch granules will begin to swell till they reach four to five times their original volume, while wheat starch is scarcely affected. In order to render this test sharper, it may be combined with Lecanu's subsidence process, when it will be possible to detect as little as one part of potato starch in a thousand of wheat flour.

The process just alluded to depends on the fact that potato starch has a higher specific gravity than wheat starch, and will consequently sink in water sooner than the latter. One hundred grams of the flour are made into a dough with water, and the gluten is separated by kneading in water; the wash water is collected, stirred and passed through a sieve to separate the coarser suspended matter, and then allowed to stand in a conical flask until a deposit has formed. The supernatant liquor is decanted while still turbid, and the deposit stirred up with more water and allowed to stand for a short time. After decanting the turbid supernatant liquor, the process is repeated once more, when the lowest portion of the final deposit will consist entirely of potato starch, if this be present. The influence of dilute potassium hydroxide solution on the starch grains may then be studied by means of the microscope, as directed above.

In bread the starch granules lose much of their characteristic appearance owing to gelatinisation on baking, but potato starch may be recognised by the readiness with which it takes up such stains as methylene blue or neutral red.[1]

Leguminous Starches.—Leguminous starches may be detected with comparative ease in wheat flour by microscopic examination (see p. 307). If a thin layer of the flour be mounted in a mixture of water and glycerol, in the proportion of 2 parts of the former to 1 of the latter, and examined under the microscope between crossed Nicol prisms, a selenite plate being interposed between the object and the lower Nicol prism, the leguminous starches will show no play of colours, and will easily be detected among the iridescent starch granules of wheat. Further, if the flour is treated under the microscope with a 10 to 12 per cent. solution of potassium hydroxide, it will be possible to dissolve the starch of the leguminosae, leaving a characteristic reticular tissue, made up, for the most part, of irregular hexagons.

Separation of Legumin.—Leguminous starches may be detected by the separation of legumin, a constituent peculiar to this group. Lecanu's process is as follows: The gluten is separated in the usual manner, as described above under the gluten test, the wash water, which contains the starch, soluble matter and legumin, being collected, passed through a sieve to separate the coarser suspended matter, diluted, if necessary, and allowed to settle. The supernatant liquid is divided into two parts; one of these is allowed to putrefy or ferment spontaneously; with pure wheat flour, lactic acid fermentation is the most common; with flours containing legumin, putrefactive fermentation will set in at once. In lactic acid fermentations, the principal change which occurs is the transformation of sugar into lactic acid, a change which may be followed by noting the increasing acidity of the liquid; in putrefactive fermentations, the proteins are decomposed. (See the account of the decomposition of milk by micro-organisms in Chapter VI.)

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[1] Schütz and Wein. Chem. Zeit., 1915, 39, 143, abs. *Analyst*, 1915, 235.

The second portion of the aqueous extract from the flour is filtered clear, and concentrated by evaporation until a yellowish scum forms on the surface; it is then allowed to cool, and filtered in order to remove the albumen, which, at this stage, will have separated from the flour whether it contains leguminous products or not. The legumin, if present, is precipitated from the filtrate by the addition of a drop of acetic acid, filtered off and dried in the steam oven. When dry, legumin is of a horny consistence, insoluble in alcohol, not coloured by iodine and easily soluble in caustic alkali or ammonia solutions, from which it may be precipitated by the addition of acid. According to Lemenant des Chenais, 0·9 parts of legumin in 100 parts of flour, represents an adulteration corresponding to 5 per cent. of added leguminous flour. Too much reliance should, however, not be placed on a quantitative estimation of this kind.

Rice Flour.—Rice flour may be detected in wheat flour by a microscopic examination of the starch granules (see p. 306). Wheat starch, in common with the starches of rye and barley, shows no hilum or concentric rings in the majority of granules, which are nearly circular, and flattened in shape. The granules of rice starch, on the other hand, are polygonal in shape, as viewed under the microscope, show faint rings, and under high magnification, a starred hilum. Gastine advises staining with aniline blue or green (as previously described), which will show up the hilum of the minute starch granules as a reddish point.

In all these microscopic examinations, comparison should always be made with samples which are known to be genuine, as well as with samples which have been purposely adulterated.

THE EXAMINATION AND ANALYSIS OF BARLEY.

Barley may vary in colour from whitish yellow to brown, the best usually being that of a bright yellow colour. If too light, the grain may have been bleached by chemical means (sulphurous acid), while if too dark, or coloured brown at the tips, it has probably been unduly exposed to rain. It should possess a sweet smell like that of straw; if musty or mouldy in smell, it has probably been badly harvested or stored. The various outward characteristics by which the expert judges the quality of barley will not be detailed here, and the mechanical tests will only be briefly referred to. The student desiring further information on these points, is referred to the works on the subject of brewing, mentioned at the end of the present chapter.

Sampling.—Several portions are taken from different places and depths in the sack or heap, and well mixed; 500 grams are then taken as a sample for analysis, which is preserved in an air-tight vessel.

Mechanical Tests.—The more important mechanical tests are as follows: (*a*) Determination of *glumes or chaff*, which should not amount to more than about 7 to 10 per cent. of the grain (Weber). (*b*) *The form and size* of the corns may be examined by specially constructed sifting machines; (*c*) the "*Sinker*" *Test*; when several hundred corns are thrown into water, only about 2 to 3 per cent. should float; (*d*) *the relative transparency* of the grain may be observed by means of apparatus specially designed for the purpose; (*e*) *the weight of* 1000 *corns* (air dried).

Germination Test.—About 500 corns are placed at the ordinary temperature on moist blotting paper between glass plates and observed after three days and six days. They should all germinate at practically the same time.

CHEMICAL EXAMINATION OF BARLEY.

Water.—The Institute of Brewing method for determining the moisture content in barley is as follows: About 10 grams of the sample are ground as finely as possible in a small coffee mill, a little of the sample having been put through the mill beforehand. Four to 5 grams are then weighed accurately in a weighing bottle provided with a stopper, or in a beaker or crystallising dish provided with a watch glass cover. The vessels should be 2 inches in diameter. The samples are dried in a boiling water oven for 5 hours, while the stoppers or covers are kept on the top of the oven. The closed vessels are transferred to a desiccator and weighed as soon as possible after cooling. Several precautions are given: Nothing else but barleys should be in the oven, which must be kept ventilated, the door being kept closed during the period of drying. Products of combustion from the burners should be prevented from entering the oven. The samples should be placed on the bottom of the oven, away from the door, and the shelf of the oven should be out of use. Further, it is advised that distilled water should be used in the oven to prevent furring, and that a control determination should be made on a stock sample of barley with each batch. The results should be accurate to within 0·5 per cent.

The water percentage in barley, which may vary from 12 to 18, is an important factor in its commercial valuation; a good barley should contain from 13 to 15 per cent. of water.

Fat.—The dried residue from the water determination is extracted with ether in the Soxhlet apparatus. (See p. 87.)

Barley may contain from about 1·6 to 2·6 per cent. of fatty matter. Occasionally, however, the barley may have been treated with oil in order to give it a shiny appearance, in which case the fat content as determined by the above method will be materially increased.

Nitrogen (*Proteins*).—This is determined on 2 to 3 grams of barley by the Kjeldahl-Gunning process. (See p. 26.) The factor for converting nitrogen into proteins is 6·25.

According to Weber, the protein content of barley may vary from 6 to 18 per cent., but generally speaking, a good barley for brewing purposes should contain not less than 8, and not more than 11 per cent. of proteins. A low protein content affects the yeast unfavourably in the fermentation process, while with a high protein content, the amount of extract obtained from the malt is decreased.

Starch.—This constituent may be determined on the finely ground barley by the methods described on pp. 309 to 317.

The percentage of starch in barley usually lies between

70 and 80, and furnishes a measure of the proportion of extract obtainable from the malt for brewing purposes.

Ash.—This may be determined on the finely ground barley, as described for flour on p. 326.

The content of ash in barley usually lies between 2·5 and 3·5 per cent.

Test for Bleaching by Sulphur Dioxide —Mixed or badly coloured barleys may be made to assume a uniform light yellow colour by treatment with sulphurous acid.

Evidence of such treatment may be obtained as follows: (Weber) 100 grams of the barley are mixed with 100 c.c. of water, stirred at intervals for half an hour, and filtered. 100 c.c. of water and a few pieces of pure zinc are placed in a beaker, and sufficient concentrated hydrochloric acid is added to cause a moderately rapid evolution of hydrogen. The beaker is covered with a piece of filter paper, the middle of which has been moistened with a few drops of lead acetate solution. If, after some time, no brown or black stain appears on the paper, the zinc and acid are sufficiently pure, and the test may be proceeded with. The water is poured off from the zinc, and the filtrate from the barley added in its place, together with a little concentrated hydrochloric acid Sulphurous acid, if present, will be reduced to sulphuretted hydrogen by the action of the nascent hydrogen, and a brown or black stain of lead sulphide will appear on the filter paper covering the beaker. If only traces of sulphurous acid are present, the stain may not appear for about 10 minutes.

THE EXAMINATION AND ANALYSIS OF MALT.

The value of a malt extract for brewing purposes depends very largely on its diastatic activity, *i.e.*, on the ability of the diastase which it contains, to effect a rapid conversion of the starch into maltose and dextrin.

Before the determination is described, a few words may be said regarding the preparation and composition of malt. Malt is prepared by steeping barley in water and then allowing it to germinate at a suitable temperature; during this process, diastase, as well as other enzymes, are formed in order that the starch may be converted into soluble products which can be assimilated by the growing embryo. Before germination has proceeded to any extent, the vital processes are stopped by drying and curing the grain in a kiln; the action of the diastase on the starch and other carbohydrates is thereby stopped for the time being, to be continued when the malt is subsequently made into a mash with water. During the drying process, various substances are formed which impart colour and flavour to the malt and to the products prepared from it.

Malt varies in colour from light yellow to dark brown, according to the origin of the barley and the degree of the curing, or drying. It should be crisp and white inside; its diastatic activity depends very largely on the freshness and quality of the grain from which it is prepared, as well as on the degree of curing.

The following typical analysis, from Blount and Bloxam's "Chemistry for Engineers and Manufacturers," will give an idea of the approximate composition of barley and malt; the most noticeable differences, on the analytical figures, between barley and malt are the lower proportion of water and the higher proportion of soluble carbohydrates contained in the latter. Further, malt usually contains slightly less fatty matter, and more soluble nitrogen compounds than barley.

—	Barley.	Malt.
	Per cent.	Per cent.
Water	14·1	5·8
Proteins	10·6	13·1
Fat	2·1	1·7
Carbohydrates	63·7 (mainly starch).	65·4 (about ⅛ being fermentable sugar)
Fibre	7·1	11·6
Ash	2·6	2·6

Sampling.—See under the Examination and Analysis of Barley.

Mechanical Tests.—Tests similar to those enumerated under (*b*) to (*d*) on p. 344, for barley, are applied also to malt.

CHEMICAL EXAMINATION OF MALT.

Water.—This may be determined by drying about 5 grams of the finely powdered malt in a weighing bottle, as described for barley on p. 345.

Freshly cured malts may contain from about 1 to 3 per cent. of water, while malts which have been stored for any length of time, may contain from 4 to 6 per cent., or even more. Malts containing more than 6 per cent. of water may usually be regarded as inferior in quality, yielding beers of poor taste and keeping qualities. Pale, or lightly cured malts generally contain slightly more water than dark malts. Malts which are known to have been stored for a long time and which contain less than 3 per cent. of water should be regarded with suspicion, as they may possibly have been subjected to an extra drying.

Preparation of Extract.—An important feature in the valuation of malt for brewing purposes is the preparation of an aqueous extract for chemical and physical examination. Great care should be taken in carrying out the following directions, as the analytical results are likely to be influenced by apparently trifling differences in the method of preparing the extract. The directions, given by Weber, include a "saccharification test."

For the preparation of the mash, a 500 c.c. beaker of nickel, aluminium or brass will be required. The clean, dry beaker is first weighed together with a thermometer, and the weight noted. A little over 50 grams of the malt is ground to a fine powder, and exactly 50 grams of the powder weighed out in the beaker. 200 grams of water at 48° to 50° are added, and the beaker is immediately placed in a water bath at 45°, and kept at this temperature for half an hour; the temperature is then raised at the rate of 1° per minute up to 70°, and kept at this point for 1 hour; throughout the whole mashing process, the contents of the beaker should be gently stirred with the thermometer. After the mash has been kept at 70° for 10 minutes, a drop is removed and tested for starch by mixing with a drop of iodine solution on a white tile; if a red or blue coloration is produced, the whole of the starch has not yet been converted into dextrin and maltose, and the test is repeated every 5 minutes until no coloration is obtained with iodine.

The time from the moment at which the temperature of the mash reached 70° to the complete disappearance of the starch is noted (Saccharification Test). With pale, lightly cured malts, of high diastatic value, the time for "saccharification" is usually about 10 to 15 minutes, with intermediate malts, 15 to 20 minutes, and with dark malts, which have somewhat lower diastatic values, owing to the heavy curing, 25 to 30 minutes.

When the saccharification is complete, the material adhering to the sides of the beaker is loosened by means of the thermometer and use of a wash bottle, and the temperature kept at 70° until the hour is up. The beaker is then removed from the water bath, and 200 c.c. of distilled water are added, or correspondingly less if much water was used in loosening the material adhering to the sides of the beaker. The whole is cooled to under 20°, the outside of the beaker wiped dry, and the weight of the contents made up to exactly 450 grams with water, the weight of the empty beaker and thermometer having previously been determined as directed. The contents of the beaker are well mixed and brought on to a dry fluted filter; the filtrate, or wort, as it is called, may now be used for the determinations of extract, sugar, nitrogen, ash and phosphoric acid, and depth of colour. It is of interest from the point of view of the brewer to note the odour of the wort and whether it filters clear or turbid.

Determination of Extract.—The specific gravity of the wort at 17·5° is determined by means of the pyknometer. A float specially designed for the purpose may also be used, though the results obtained by this method are less accurate. The percentage of extract by weight may then be found by calculating the specific gravity to the basis water = 1000, subtracting 1000 and dividing by 4. (Cf. p. 369.)

It now remains to calculate the percentage of extract on the malt itself (air dried) or the anhydrous malt.

Suppose the wort to contain 8·0 per cent. of extract, and the malt to contain 5·0 per cent. of water.

One hundred parts of wort will then contain 92 parts of water. The weight of mash before filtering was 450 grams, consisting of 400 grams of water, and 50 grams of malt. As the 50 grams of malt contained 2·5 grams of water, the total water in the 450 grams of mash was 402·5 grams. Now in the water, 92 parts of wort correspond to 8 parts of extract, so in the mash, 402·5 parts of water will correspond to

$$\frac{8{\cdot}0 \times 402{\cdot}5}{92{\cdot}0} = 35{\cdot}0 \text{ parts of extract.}$$

Thus, 50 grams of malt yield 35 grams of extract, therefore the malt will yield 70·0 per cent. of extract.

As 100 parts of malt consist of 5 grams of water and 95 grams of anhydrous malt, the anhydrous malt will yield

$$70 \times \frac{100}{95} = 73{\cdot}7 \text{ per cent. of extract.}$$

The yield of extract on the anhydrous malt usually lies between 72 and 80 per cent.

It is of interest to the brewer to know the yield of extract obtainable from malt, as this, together with the added brewing sugar[1] (invert sugar or glucose) constitutes the fermentable material used in the brewing of beer. The yield is usually stated in lbs. per quarter (28 lbs.).

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[1] Brewer's "glucose" contains, besides dextrose, maltose and dextrin.

Sugar.—A portion of the wort, obtained as described above, is diluted to ten times its volume, and the sugar determined as described on p. 373. (See also p. 374.) The copper oxide obtained may be converted to maltose by reference to the table on p. 377.

In order to find the percentage of sugar in the extract, the percentage of extract *by volume* in the wort is found as described on p. 352. Having determined the sugar in a given volume of wort, the sugar in the extract corresponding to this volume of wort is easily calculated, and hence the percentage of sugar in the extract.

Subtracting the percentage of sugar in the extract from 100, the percentage of matter other than sugar in the extract is found.

According to Weber, the ratio of the sugar to extract less sugar, varies from 1 : 0·4 or 0·5 in pale (Pilsner) malts, to 1 : 0·6 or 0·7 in dark (Munich) malts.

Nitrogen.—Twenty-five c.c. of the wort, obtained as described above, are evaporated to a syrup, and the nitrogen, and hence the proteins, determined in the latter by the Kjeldahl-Gunning process, as described on p. 26. As in the case of malt, the factor for converting nitrogen into proteins is 6·25.

The percentage of nitrogen, calculated on the extract, varies from 0·5 to 0·8, and the proteins from 3 to 5.

The nitrogen may also be determined direct, on the finely ground malt.

Although both in barley and malt the whole of the nitrogen is not present as proteins, the determination of nitrogen is nevertheless of value when used comparatively, especially in its bearing on the physiology of the fermentation process.

Ash.—Fifty c.c. of the wort are evaporated to dryness in a weighed platinum dish, and the residue incinerated. (See p. 326.)

The content of ash in the extract (calculated as explained above for sugar) varies from 1·4 to 1·9 per cent.

Acidity of Malt.—One hundred grams of finely powdered malt are well mixed with 400 c.c. of 20 per cent. alcohol, and allowed to stand for 6 hours, the mixture being shaken from time to time. The mixture is then filtered, and 100 c.c. of the filtrate are titrated with decinormal sodium hydroxide or baryta solution, until a drop brought on to a piece of sensitive blue litmus paper no longer produces a red coloration. The number of cubic centimetres of decinormal alkali used is calculated to lactic acid by multiplying it by 0·009.

The percentage of acid, calculated as lactic acid, in malt, varies from 0·15 to 0·4.

Determination of the Diastatic Activity (*Lintner Value*) *of Malt.*[1]—This determination is based on Kjeldahl's observation that in malt diastase conversions, where the amount of maltose formed does not exceed 45 per cent. of the starch originally present, the amount of maltose produced in a given time may be taken as a measure of the activity of the diastase solution used. The actual method described is due to Lintner. In the first place, the preparation of soluble starch and the malt extract will be described.

Preparation of Soluble Starch.—This may either be obtained ready-made or prepared as follows: 100 grams of purified potato starch are digested with 500 c.c. of dilute hydrochloric acid of specific gravity 1·037, at the ordinary temperature, for 7 days, the mixture being stirred daily. The mass is then washed repeatedly by decantation, first with tap water and then with distilled water, till all the acid has been removed. When the moist starch gives no acid reaction when spread on blue litmus paper, a few drops of dilute ammonia are added, and the starch again washed by decantation until all the ammonia has been removed. It is then collected on a Buchner funnel, sucked as dry as possible, spread on a porous plate and dried at a gentle heat (about 30°). When required for use, the pulverised starch is dissolved in boiling water to make a 2 per cent. solution which, on cooling, should be perfectly mobile, and not gelatinous, as would be the case with a solution of untreated starch. Its action on Fehling's solution, on boiling, should be negligible.

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[1] See also the Saccharification Test (p. 350).

Preparation of Malt Extract.—Twenty-five grams of the finely ground malt are extracted with 500 c.c. of distilled water for 3 hours at 21°, agitating the mixture from time to time. The whole is then filtered, the first 100 c.c. of the filtrate being rejected. The extract obtained should be perfectly clear and bright.

The actual determination of diastatic activity is carried out as follows: Portions of 10 c.c. of a 2 per cent. solution of soluble starch are measured out in eight carefully cleaned test tubes, which are placed in a suitable stand and immersed in a water bath kept at 21°. When the starch solution has reached the temperature of the bath, 0·1 c.c. of the malt extract to be tested is measured into the first tube, 0·2 c.c. into the second, 0·3 into the third, and so on, to the eighth tube, into which 0·8 c.c. will be introduced. In examining pale malts, *i.e.*, malts which usually have a relatively high diastatic power owing to the lightness of the curing, it is recommended to dilute the extract obtained as directed above with an equal volume of water, and to add it to the starch solution in the tubes, in amounts of 0·2 c.c., 0·3 c.c., 0·4 c.c., etc.; in this way, greater accuracy will be attained. The tubes are allowed to remain in the water bath at 21°, for exactly one hour from the time the extract has been added. Five c.c. of mixed Fehling's solution (see p. 373) are then added to each tube, and after shaking the tubes are heated in boiling water for 10 minutes and allowed to stand until the cuprous oxide has settled. Usually the liquid in one of the tubes is faintly blue, showing that the maltose present was insufficient in amount to effect the complete reduction of the cupric salt present in the 5 c.c. of Fehling's solution, while the liquid in the next tube of the series is yellow, owing to over reduction. In this way the amount of malt extract, which is just sufficient to produce, in the given time, the quantity of maltose to reduce 5 c.c. of Fehling's solution, may be estimated. Thus, if tube No. 2 is as much under reduced as tube No. 3 is over reduced, then the amount of malt extract sufficient to cause complete reduction may be taken at 0·25 c.c. (*i.e.*, if tube No. 2 contained 0·2 c.c., and tube No. 3 contained 0·3 c.c. of the extract). If the liquid in one of the tubes is neither blue nor yellow, then the maltose formed therein will have been just sufficient to cause complete reduction.

Complete reduction by 0·1 c.c. of malt extract corresponds to a Lintner value of 100. If X is the quantity of malt extract required for complete reduction, in cubic centimetres, then the diastatic activity of the malt,

$$A = \frac{0{\cdot}1 \times 100}{X}.$$

From the figure thus found, it is usual to deduct 1·5, to allow for the reducing sugars present in the malt extract. If the extract has been diluted with an equal quantity of water, then half the volume actually added must be used for the purposes of the calculation.

Diastatic activities of over 80 are considered high, under 50 low.

The modification of the above method adopted by the Institute of Brewing is as follows: The details are as described above, excepting that 100 c.c. of the soluble starch solution are treated with 3 c.c. (more or less according to the diastatic activity) of the 5 per cent. extract to be tested. After 1 hour at 21°, the action is stopped by the addition of 10 c.c. of decinormal soda solution, and the whole made up to 200 c.c. The amount of maltose present is determined by titration with Fehling's solution. When 0·1 c.c. of the 5 per cent. extract produces enough maltose (0·04 gram) to reduce completely 5 c.c. of Fehling's solution, the material is said to have a diastatic power of 100. If 0·2 c.c. are required, the diastatic power is 50, and so on.

Colour of the Wort.—The depth of colour of the wort, obtained as described above, is determined by means of specially designed colorimeters, comparison being made with the colour of iodine solutions of known strength.

INFANTS' FOODS.

In the previous chapter only preparations made from milk were considered. From Dr. Coutts' Report (footnote 2, p. 311) it will be seen that there are many foods on the market for which extravagant and misleading claims are made. Such are those which contain large amounts of starch, being chiefly composed of flour, *e.g.*, wheat or oatmeal which may or may not have been baked. As starch is unsuitable as food for young infants, at any rate up to the age of six months, the present position regarding the sale of infants' foods is unsatisfactory in view of the ease with which the public may be misled by printed statements. Some of the preparations contain small amounts of diastase due to the addition of a malted cereal; but if, as is often the case, the directions for preparation include the use of boiling water, the diastase will be destroyed. In some of the preparations the starch has been partly or wholly hydrolysed to maltose, malto-dextrin or dextrin by the action of diastase. Cane sugar, lactose or dried milk may be found in the starchy foods.

The analysis of these preparations is by no means a simple matter, and if it is desired to make a thorough investigation of the subject, reference should be made to Mr. Julian Baker's Report (footnote 2, p. 311). In what follows, only a few of the main points contained in this Report are given.

Starch.—For the detection of starch and determination of its origin, see p. 304. If the preparation has been heated, the starch granules will be distorted as in bread. The method used by Mr. Baker for the estimation of starch is given on p. 311. The taka diastase method might also be used, but, as pentosans are absent or practically absent from the products under examination, there is no objection to the polarimetric method, provided that allowance is made for the optical activity of the sugars present. (See p. 312.)

The analyses quoted in the report show a large proportion of samples containing from about 60 to 70 per cent. of starch. Such samples would consist mainly or entirely of flour. Wheat starch was found in the majority of cases. Oat, lentil, banana, arrowroot and other starches were also found.

Hydrolysed Starch Products.—These products (see above) were not determined directly, as no simple method is available, but they were calculated by difference from the specific gravity, reducing power and optical activity of the cold water extract of the sample, after other carbohydrates, proteins, etc., had been determined. (See p. 395.)

Water Extracts.—Ten grams of the sample were extracted for 3 hours in 200 c.c. of distilled water at 15·5° with frequent stirring. In cases where the fat exceeds 5 per cent., it is advised to use the fat free material. The filtered extract was used for the estimation of sugars and starch conversion products if present. For the purpose of calculating the amount of the latter, the soluble proteins were also determined. At the same time, the amount of matter soluble in water at 35° under the same conditions was also determined. Differences in solubility were due either to the action of diastase during the extraction at the higher temperature, or to the fact that the preparation had been heated so as to render the starch soluble at this temperature. In the absence of diastase, as determined by the Lintner value, an idea could be formed as to the extent to which the starch had been altered by heat. Thus, if no difference was observed, the preparation had not been heated; in many cases a difference of about 1 to 3 per cent. was observed, indicating that the starch had been slightly altered by heating.

Sugars.—For the estimation of these, reference may be made to Chapter VIII. As mentioned above, they were determined in the filtered cold water extract. Lactose, cane sugar and other reducing sugars, as dextrose, were determined. The last mentioned were returned in the absence of products of starch hydrolysis; they include the small amounts of sugars natural to the flour (usually dextrose and lævulose) in amounts under 2 per cent. Cane sugar was found in amounts up to about 12 per cent. The presence of lactose would be an indication of the presence of dried milk, which would be confirmed by the composition of the preparation, especially as regards the percentages of proteins, ash and fat. On the other hand, lactose only may be added, in which case the figures just referred to would not be sensibly affected (see below).

Other Constituents.—Fat may be estimated, as in milk powder, by the Gottlieb method (p. 263). *Moisture* and *Ash* may also be estimated as described for milk powder (pp. 280 and 296). *Proteins* may be estimated by the Kjeldahl-Gunning method (p. 26). As in the case of the fat, no distinction is made in respect to the origin of the proteins, but if the preparation has been made on a basis of flour, the factor 6·25 should be used for converting nitrogen to proteins. *Diastatic Power* may be determined by the method given on p. 357.

A comparison of the compositions of dried milk and wheat flour (pp. 295 and 320) will show the significance of the analytical results in determining the general nature of a preparation. Thus unchanged flour may be taken as containing roughly 70 per cent. of starch. Hydrolysed starch products are the main constituents of dried malt extract, but may also have been produced by the action of diastase on flour. Lactose is characteristic of milk powder, being the only carbohydrate contained in the genuine product. In deciding whether a product contains added lactose or milk powder, account may be taken of the facts that milk powder contains about twice as much protein matter as wheat flour, about twelve times as much mineral matter and (whole) milk powder about twenty-five times as much fat. Oat flour, however, is far richer in fat than wheat flour (cf. table, p. 318); thus Mr. Baker quotes an analysis of a preparation consisting entirely of oat flour, showing 7·8 per cent. of fat. Wheat and oat flours contain about 1 or 2 per cent. of reducing sugars other than lactose, while small amounts of cane sugar, of the order of 1 or 2 per cent., are found in these flours as well as in dried malt extract. Only larger amounts will be due to added sugar in flour preparations. The diastatic value will give an idea as to whether it can justly be claimed that the preparation is "malted."

Cocoa.

As several methods applicable to the analysis of cocoa have already been described, a few words may be said on this subject.

Starch in cocoa has been treated of under Microscopic Examination and the Taka-Diastase Method (pp. 306 and 314). For *Fat*, see p. 115; for *Nitrogen*, see the Kjeldahl-Gunning method (p. 26); and for *Fibre*, see p. 328. *Moisture* and *Ash* may be determined on the same lines as in milk powder (see pp. 280 and 296). Fibre, starch and ash should be determined on the fat free material. *The Water Soluble Extract* is determined by shaking up 2 grams of the fat free material with 100 c.c. of cold water till no lumps remain, allowing to stand overnight, and then filtering till a perfectly clear filtrate is obtained.

Fifty c.c. of the filtrate are evaporated, dried and weighed.

Among other methods used in the analysis of cocoa are those for the estimation of the proportion of *shell* to nib. In the microscopic examination the particles of shell are observed as reddish brown particles of varying size and shape which stain brightly on the addition of a small quantity of ruthenium red when mounted in lead acetate solution. With a good deal of practice a rough idea of the amount of shell present may be formed, but naturally this method is somewhat uncertain. The subject is too complicated to be adequately discussed in a small space. For further information, reference may be made to the recent publications of Baker and Hulton, and Knapp and M'Lellan,[1] where the various methods available are dealt with.

The results obtained by the above methods may vary greatly with different samples, owing chiefly to variations in the proportion of shell to nib, and in the amount of fat left in the product in the process of manufacture, as well as the addition of starch, etc.

As regards starch, only such figures as have been obtained by the taka diastase method are reliable (see p. 313). By this method Revis and Burnett found from 8·0 to 14·5 per cent. of natural starch in the fat free dry nib, and none in the shell. König found an average of 49 per cent. of fat in the nib bean, but the proportion in commercial cocoas is usually much lower. The percentages of nitrogen and fibre, especially the latter, may give some indication as to the proportion of shell (see footnote). The amount of cold water extract in the fat free dry material should be about 24 per cent., which figure will be raised or lowered according as soluble or insoluble matter has been added to the cocoa.

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[1] *Analyst*, 1918, 197, and 1919, 2 (including discussion).

FEEDING STUFFS.

Several methods have been described which are applicable to the analysis of feeding stuffs for cattle. These may be enumerated here.

Moisture and *Ash* may be determined on the same lines as described on pp. 280 and 296. For the estimation of *Fat*, see p. 115. *Proteins* may be determined by the Kjeldahl-Gunning method (see p. 26), using the factor 6·25 for calculating the proteins from the nitrogen content. For the determination of *Crude Fibre*, see p. 328. Starch is often estimated by a polarimetric method (see p. 310), but it is probable that the taka diastase method would be better. Other estimations are those of soluble and amide nitrogen, phosphoric acid and potash, for which other works may be consulted.

REFERENCES.

"Foods, their Composition and Analysis," by A. and M. Wynter Blyth. 1909. Chas. Griffin & Co., London.

Allen's "Commercial Organic Analysis" (revised edition), Vols. I. and VIII. 1909. J. & A. Churchill, London.

"Technical Methods of Chemical Analysis," by Lunge, translated by Keane. 1911. Gurney & Jackson, London.

"Chemisch-Technische Analyse," Vol. II., by Post. 1908. Fr. Vieweg, Brunswick.

"Anleitung zu den Chemisch-Technischen Untersuchungsmethoden für Bräuer," by F. Weber. 1904. J. L. Stich, Nürnberg.

"Laboratory Studies for Brewing Students," by A. J. Brown. 1904. Longmans, Green & Co., London.

"The Brewer's Analyst," by Douglas Bailey. 1907. Kegan, Paul, Trubner & Trench, London.

"The Student's Manual of Yeast Culture," by Grove Johnson. Published by the Author, London, 1908.

"Brewing," by Chaston Chapman. 1912. Cambridge University Press.

"The Principles and Practice of Brewing," by Sykes & Ling. 1911. Chas. Griffin, London.

Leach's "Food Inspection and Analysis." Third edition. By A. L. Winton. 1914. John Wiley, New York.

"Applied Analytical Chemistry," by Villavechhia and others, trans. by T. Pope. 1918. J. & A. Churchill, London.

"Organic Chemistry, General and Industrial," by Molinari, trans. by T. H. Pope. 1913. J. & A. Churchill, London.

"The Microscopical Examination of Foods and Drugs," by H. G. Greenish. 1903. J. & A. Churchill, London.

"Compendium of Food Microscopy," by G. Clayton. 1909. Balliere, Tindall & Cox, London.

"The Microscopy of Vegetable Foods," by Winton and Moeller. 1916. John Wiley, New York.

"Elementary Chemical Microscopy," by E. M Chamot. 1915. John Wiley, New York.

CHAPTER VIII

THE SUGARS AND ALCOHOL

PART I.—THE SUGARS

INTRODUCTORY

THE sugars form a well-defined group of plant and animal products of great physiological importance. As the fundamental members of the carbohydrate group, they may be regarded as representing the simplest recognisable group of stable products in the synthesis of the complex carbohydrate material of plants which has its starting point in that remarkable interaction between carbon dioxide and water in the presence of chlorophyll and light, which is still so imperfectly understood. It is in the form of sugars that the carbohydrate material is distributed through the vascular system of the plant; temporarily, sugar may be converted into starch or other insoluble material such as fat, and stored as reserve food material until such time as it may be required in other parts of the growing plant, or, in the case of seed and tubers, etc., by the embryo plant. In animal nutrition the importance of the sugars is well known. In the digestive processes sugar is formed by the hydrolysis of the more complex carbohydrates, notably starch, for assimilation by the organism. Milk sugar or lactose is an important and characteristic constituent of cow's milk. It is also found in the milk of other mammals, but in some milks other little investigated sugars have been found. Industrially, some of the sugars are of importance as the source of alcohol by fermentation with yeast.

The great resemblance which many of the members of the sugar group bear to one another, and the subtle differences in chemical constitution which they display, render this branch of Analytical Chemistry at least as difficult as that dealing with the fatty oils and fats. The proper understanding of the "purely chemical" side of the subject is important at all times, but in the case of sugar analysis a thorough study of theoretical foundations is especially necessary if the analytical processes are to be understood, *i.e.*, understood as far as our present knowledge of the subject permits.

In this chapter only some of the more well-known sugars will be dealt with, and in some cases the processes described are supplementary to processes described in other chapters. The determination of lactose in milk by one method has already been described in Chapter VI., and the determination of pentoses, *i.e.*, sugars containing five carbon atoms in the molecule, in Chapter VII. It will be necessary to assume a knowledge of the chemistry of sugars from text-books of Organic Chemistry or special works on sugars, though explanatory remarks will be offered in the course of the description of the processes in order to direct attention to certain points. The sugars dealt with are all hexoses, *i.e.*, sugars containing six carbon atoms in the molecule or such as are resolvable into hexoses by hydrolysis.

MAIN METHODS USED IN SUGAR ANALYSIS.

Total Sugar in Solution.—This may be determined by calculation from the specific gravity of the solution, provided that any extraneous matter which may be present, such as proteins or gummy matter, has been removed. The specific gravity may be determined by the usual methods, using a pyknometer, specific gravity bottle, Westphal balance (p. 50), or special hydrometer. In practice some form of the last mentioned instrument is often used; the Brix and the Balling saccharometers are graduated so as to give directly the percentages of cane sugar at 17·5°. Bates' brewer's saccharometer gives the number of "pounds per barrel" of extract in malt worts (see p. 352). For the present purpose, the specific gravity should be taken at 15·5°. In sugar work the results are often stated as 1000 times the specific gravity. Thus, a specific gravity of 1·0389 would read 1038·9. The strengths of carbohydrate solutions may be calculated in grams per 100 c.c. by dividing the specific gravity at 15·5°, with reference to water at the same temperature, less 1000, expressed as explained above, by a solution factor which only varies slightly for the different carbohydrates, and which may be assumed

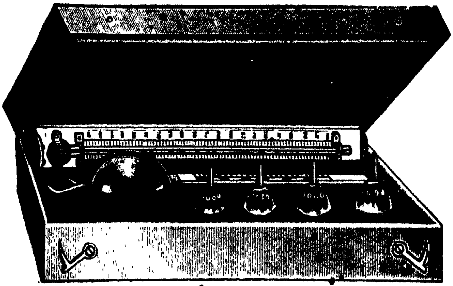

FIG. 25.—Bate's Saccharometer.

to be 3·86 for all carbohydrates without great error. Examples are given below. The method is most accurate when applied to solutions of 10 per cent. strength or under.

Copper Reduction Method.—The reducing properties shown by most sugars is perhaps one of the best known and least understood reactions in Organic Chemistry. The old aldehydic or ketonic formulae, which apparently provided a simple explanation of this behaviour, certainly fall far short of being a representation of the whole truth regarding the constitution of the sugars, as far as structural formulae can be said to do this. For quantitative work, Fehling's alkaline tartrate copper solution is the most generally used. The reduction of Fehling's solution by sugars cannot be expressed by definite chemical equations, and its quantitative applications are based on empirical data. The solutions must be prepared of definite strength from pure chemicals, mixed immediately before use, and the reduction must be carried out under standard conditions. The only common sugar which does not reduce Fehling's solution is cane sugar, or sucrose, a fact which is sometimes explained by saying that this sugar, unlike the other common sugars, has no "free carbonyl group in the molecule." Cane sugar is a disaccharide, and is easily hydrolysed by the action of dilute acids, or the enzyme invertase, into the monosaccharide hexoses, dextrose or dextro glucose and lævulose or dextro fructose. The resulting mixture, known as invert sugar, reduces Fehling's solution, so that it is possible to estimate sucrose in presence of other sugars by the increase in reducing power after inversion. The polysaccharide dextrin, like starch, does not reduce Fehling's solution, but gives rise to reducing sugars on hydrolysis (cf. p. 381).

Two main methods are given for the determination of reducing power. (*a*) The cuprous oxide is collected and estimated by some gravimetric or volumetric process. (*b*) The sugar solution is titrated with Fehling's solution. Of the various volumetric methods which have been proposed from time to time, that given below is the only one which can be recommended.

The Polarimetric Method.—As all sugars are optically active, the polarimeter plays an important part in their identification and estimation. In many cases it is necessary to subject the solution to a process of clarification before the polarimetric reading can be taken; see, for example, the method described for milk on p. 391. The particular method employed depends on the nature of the solution and the sugars present. Animal charcoal and basic lead acetate are very commonly used, the exact methods being described below.

A factor to be taken into account when dissolving solid sugars for polarimetric observation is mutarotation.[1] Most sugars exist in two forms, designated α and β, which have different optical activities, and on dissolving in water a change takes place which results in the formation of a definite equilibrium mixture between the two forms. The change is explained by means of the oxidic structural formulae for sugars, as being due to the interchange of positions of a hydrogen atom and a hydroxyl group. All figures used in sugar analysis apply to the equilibrium mixture of constant rotation. The change is completed spontaneously on standing for about twelve hours, or more rapidly by boiling the solution for a few minutes or adding a trace of ammonia.

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[1] This phenomenon was first studied systematically by E. F. Armstrong and Lowry, *J.C.S.*, 1903, 83, 1305 and 1314; 1899, 75, 212.

Various types of polarimeter have been constructed for the estimation of sucrose in industrial practice. A "normal weight" of the product, varying with the particular instrument used, is dissolved in water and, after clarifying if necessary, made up to 100 c.c. at 20° C. The percentage of sucrose is read off from the scale divisions. The figures used below are in angular degrees, but if any of the special instruments referred to is used, the scale divisions may be converted into angular degrees by a special factor. It is assumed that sodium light is used. Readings are usually taken at 20° C. and sometimes at 17·5° C. Attention must be paid to the calibration of the measuring flasks used so that the correct volume is obtained at the temperature employed. The "Mohr c.c." is based on the volume of water at 17·5°. Various other precautions are mentioned in the actual description of the processes below. In the case of sucrose, the change of rotation on inversion (see above) may be made use of in estimating this sugar in the presence of monosaccharides or a disaccharide, such as lactose, which is not hydrolysed under certain conditions which secure the inversion of sucrose. In this connection it may be explained that the term "inversion" is specially applied to the case of sucrose, as a change of sign in optical rotation is involved owing to the strong lævo rotation of lævulose. The last mentioned sugar is sometimes referred to as dextro fructose although lævorotatory, for the reason that it is genetically related to dextro glucose or dextrose. The term "invert sugar" is applied to the products of the inversion of sucrose.

Other Methods.—The method recently published by Willstätter and Schudel[1] for the *estimation of dextrose*

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[1] Ber., 1918, 51, 780, abs. *J.S.C.I.*, 1918, 37, 556 A.

by means of sodium hypoiodite may prove useful, as it discriminates between aldoses on the one hand and sucrose and ketoses on the other. The latter group is not affected under the experimental conditions. Dextrose is oxidised to gluconic acid, and the amount of iodine used up in this process may be estimated by titration.

Biological methods are often of great service in the analysis of mixtures. Chemically, the sugars often resemble one another to such a degree that many analytical problems which may arise are practically insoluble unless other methods of attack are sought. It is well known that optical activity as measured by the polarimeter reflects subtle differences in molecular structure, but biological methods often afford sharper distinctions in such cases. The selective action of yeasts on sugars has been successfully employed, the fermentable sugar being destroyed by conversion into carbon dioxide and alcohol, so that the remaining non-fermentable sugar can be dealt with by itself. E. F. Armstrong [1] has shown that the monosaccharides dextrose, lævulose (invert sugar) and mannose are fermentable by all yeasts. In the case of the disaccharides sucrose, lactose, maltose and melibiose, or the trisaccharide raffinose, no action takes place unless a yeast is employed which contains an enzyme capable of hydrolysing these sugars with the formation of monosaccharides. Thus, ordinary yeast, *Saccharomyces cerevisiae*, contains the enzyme invertase which hydrolyses sucrose but not other sugars, and maltase which similarly has a selective action on maltose. Here we find distinctions which cannot be obtained by the use of acids as hydrolysing agents, though it is true that sucrose may be inverted by citric acid solution while lactose is left unaltered. An example is given below in which ordinary yeast is employed in the inversion and fermentation of sucrose in the presence of lactose. If the method is to be applied to a mixture of dextrose and maltose, it will be necessary to select a yeast which contains no maltase, such as *S. marxianus*, *S. exiguus* or *S. anomalus*.[1] In an enzyme action pure and simple an antiseptic is added (usually toluene) to exclude the action of micro-organisms (cf. p. 315); but, on the other hand, if it is desired to take advantage of the action of micro-organisms, the solution must contain no substances of an antiseptic nature, and must, moreover, not be too strong; concentrations up to 10 per cent. may be used. Yeasts grow best at temperatures in the neighbourhood of 25° in neutral or faintly acid, but not alkaline, solution. Further details are mentioned below.

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[1] *Proc. Roy. Soc.*, 1905, 76 B, 600.

Qualitative Tests.—Colour reactions of the sugars will be found in many text-books on sugars and qualitative analysis. Some of these are referred to below. Sugars are sometimes identified by the melting points of their osazones, which may be prepared by heating ½ gram of the sugar dissolved in 5 c.c. of water with 2 to 3 c.c. of phenyl hydrazine and 3 c.c. of 50 per cent. acetic acid in boiling water for ten minutes to half an hour and allowing to cool. Dextrose and lævulose yield the same osazone. Sucrose yields no osazone, and does not reduce Fehling's solution unless it has been previously inverted.

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[1] Yeast fermentation methods are described by Davis and Daish, *Journ. Agric. Sci.*, 1913, 5, 437; and Baker and Hulton, *Analyst*, 1910, 35, 512.

STARCH CONVERSION PRODUCTS.

(*A*) *Dextrose* (*by Fehling's Solution*).

The simplest example of a starch conversion product is that obtained from the process described on p. 309, in which the starch has been wholly converted into dextrose, except the small portion which has been destroyed by the hot acid.

Volumetric Method.—The method to be described is that of Ling and Rendle.[1] It is stated by Davis and Daish to be preferable to the gravimetric process in all respects, being accurate to within 0·3 per cent., as compared with 0·3 to 0·5 per cent. by the gravimetric method. It is more rapid, and has the advantage that it is standardised by the analyst himself. Like the gravimetric method, it requires some practice before accurate results can be obtained.

The following solutions are required: *Ferrous thiocyanate indicator.* One gram of ferrous ammonium sulphate and 1 gram of ammonium thiocyanate are dissolved in 10 c.c. of water, heating to about 45° to 50°, and cooling as soon as possible. Before use, the brownish red colour, due to small amounts of ferric salt, is removed by the addition of a trace of zinc dust. After several reductions, the delicacy of the solution as an indicator is impaired. *Fehling's solution.*—69·278 grams of crystallised copper sulphate are dissolved in water and made up to 1 litre. 346 grams of Rochelle salt are dissolved in hot water; 142 grams of pure caustic soda are dissolved separately in water; these two solutions are mixed and made up to 1 litre. Immediately before use, equal amounts of the copper and the alkaline tartrate solutions are accurately measured and mixed in a dry flask.

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[1] *Analyst*, 1905, 30, 182.

Ten (or 20) c.c. of the mixed Fehling's solution are accurately measured into a 200 c.c. flask and raised to the boiling point. The sugar solution, which should be adjusted so that 20 to 30 c.c. are required to reduce 10 c.c. of the Fehling's solution, is run in in small amounts commencing with 5 c.c., while the liquid in the flask is kept boiling so as to exclude the influence of atmospheric oxygen, and shaken by a rotary motion after each addition. The ferrous thiocyanate indicator is distributed in drops on a white tile, and towards the end of the titration a drop of the liquid in the flask is removed by a glass rod and mixed with a drop of the indicator. The end point is reached when, after boiling for ten seconds, no red coloration is produced, owing to the complete reduction of the copper salt. The first titration may only give approximate results, and a second or third titration may be required. The process should occupy about two to three minutes.

At the time of making the actual determination, the Fehling's solution is standardised against a solution of known strength of the pure sugar under similar conditions. The method is applicable to any of the reducing sugars. Thus it may be used for the estimation of maltose in ***malt extract,*** reducing sugar (as maltose) in ***stout,*** invert sugar in ***molasses*** (before and after inversion), etc.

Gravimetric Method.—The method of Brown, Morris and Millar[1] is as follows: The Fehling's solution is similar to that described under the previous heading, except that 130 grams of pure caustic soda are used instead of 142 grams. The amount of sugar solution taken should be regulated so that from 0·15 to 0·35 gram of copper (or the corresponding amount of copper oxide) is weighed. For this, 10 to 30 c.c. of a 1 per cent solution of the sugar will be required, and the strengt may be determined by a rough titration on the line described under the preceding heading.

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[1] *J.C.S.*, 1897, lxxi, 278.

Twenty-five c.c. of each of the Fehling solutions ar accurately measured into a 200 c.c. beaker, and th mixture is diluted with hot, well-boiled water, so that after the addition of the sugar solution, the total volum will be 100 c.c. The beaker is covered with a cloc glass and heated in boiling water for six minutes; th solution should remain quite clear. The sugar solutio is then added, and the heating continued for exactl 12 minutes, after which time the precipitated cuprou oxide must be filtered off as rapidly as possible. Th solution should still be blue, indicating excess of coppe To determine the *copper as* CuO, the precipitate i filtered off on a Gooch crucible, washed thoroughly wit hot water, then with alcohol and ether, and drie Ignition to convert the precipitate into CuO is accomplished by placing the Gooch crucible in an ordinar crucible and heating over a Bunsen flame. The asbest used in the Gooch crucible should be boiled with 20 pe cent. caustic soda solution (followed by washing wit water, dilute acid and water), for otherwise an er may be introduced owing to its partial solubility i the caustic Fehling solution. To avoid this and othe errors, however, it is best to conduct a blank experime with Fehling's solution as in the actual test, omittin only the sugar, and to make a corresponding correctio in the amount of CuO weighed.

The copper may be determined by a variety of oth methods. Thus the cuprous oxide may be weighe direct if dried at a low heat in the water oven, or it ma be filtered off in a special tube by suction, reduced to copper by heating in a current of hydrogen, and weighed as copper. Again, the cuprous oxide may be dissolved in dilute nitric acid, and the copper determined by electrolytic deposition, or the cuprous oxide may be dissolved in ferric alum solution, which is then titrated with standard potassium permanganate solution. The latter methods eliminate errors owing to the carrying down of alkaline earths with the cuprous oxide.

The following tables may be used for finding the amount of sugar corresponding to the copper precipitated according to the above directions :—

TABLE FOR FINDING AMOUNTS OF DEXTROSE, AND INVERT SUGAR, CORRESPONDING TO GIVEN WEIGHTS OF COPPER, OBTAINED BY THE METHOD OF BROWN, MORRIS AND MILLAR.

Dextrose Grams.	Copper Grams.	Dextrose Grams.	Copper Grams.	Invert Sugar Grams.	Copper Grams.	Invert Sugar Grams.	Copper Grams.
0·050	0·1030	0·155	0·3020	0·050	0·0975	0·155	0·2915
0·055	0·1134	0·160	0·3103	0·055	0·1076	0·160	0·3002
0·060	0·1238	0·165	0·3187	0·060	0·1176	0·165	0·3086
0·065	0·1342	0·170	0·3268	0·065	0·1275	0·170	0·3167
0·070	0·1443	0·175	0·3350	0·070	0·1373	0·175	0·3251
0·075	0·1543	0·180	0·3431	0·075	0·1468	0·180	0·3331
0·080	0·1644	0·185	0·3508	0·080	0·1566	0·185	0·3410
0·085	0·1740	0·190	0·3590	0·085	0·1662	0·190	0·3490
0·090	0·1834	0·195	0·3668	0·090	0·1755	0·195	0·3570
0·095	0·1930	0·200	0·3745	0·095	0·1848	0·200	0·3650
0·100	0·2027	0·205	0·3822	0·100	0·1941	0·205	0·3726
0·105	0·2123	—	—	0·105	0·2034		
0·110	0·2218	—	—	0·110	0·2128		
0·115	0·2313	—	—	0·115	0·2220		
0·120	0·2404	—	—	0·120	0·2311		
0·125	0·2496	—	—	0·125	0·2400		
0·130	0·2585	—	—	0·130	0·2489		
0·135	0·2675	—	—	0·135	0·2578		
0·140	0·2762	—	—	0·140	0·2662		
0·145	0·2850	—	—	0·145	0·2750		
0·150	0·2954	—	—	0·150	0·2832		

TABLE FOR CALCULATING THE AMOUNTS OF MALTOSE CORRESPONDING TO VARYING AMOUNTS OF COPPER.

(Method of Brown, Morris and Millar.)

Maltose, grams.	Cu, grams.	Maltose, grams.	Cu, grams.
·070	·0772	·185	·2017
·075	·0826	·190	·2072
·080	·0880	·195	·2126
·085	·0934	·200	·2180
·090	·0988	·205	·2234
·095	·1042	·210	·2288
·100	·1097	·215	·2342
·105	·1151	·220	·2397
·110	·1205	·225	·2451
·115	·1259	·230	·2505
·120	·1313	·235	·2559
·125	·1367	·240	·2613
·130	·1422	·245	·2667
·135	·1476	·250	·2722
·140	·1530	·255	·2776
·145	·1584	·260	·2830
·150	·1634	·265	·2884
·155	·1692	·270	·2938
·160	·1747	·275	·2992
·165	·1801	·280	·3047
·170	·1855	·285	·3101
·175	·1909	·290	·3155
·180	·1963	·295	·3209
		·300	·3264
		·305	·3318

Factor for converting CuO to Cu = 0·7989.

(B) *Dextrose and Maltose.*

These sugars are the sole product of hydrolysis in the taka diastase conversion; their relative proportion depends on the time of action, the final product being dextrose. The preparation of the solution for the determination of maltose and dextrose, in connection with the determination of starch by the taka diastase method, is described on pp. 312 to 317. Knowing that maltose and dextrose are the only sugars present, their relative proportion may be determined by the optical activity and the reducing power of the mixture. To determine these, it is necessary to know the concentration of total sugar in the solution.

The determination of sugar in solution by the specific gravity is explained on p. 366; the solution factor for dextrose is 3·85, and that for maltose is 3·94, but for practical purposes the factor 3·86 may be adopted without great error. A closer approximation may be got after the proportion of maltose to dextrose has been calculated from the other data on this basis. The total solid matter may be determined by evaporating a known volume of the solution to dryness, drying in the water oven and weighing. The inorganic matter may then be determined as ash by incinerating in the usual manner and deducted from the total solids.

The reducing power may be determined as described on p. 373. Taking the reducing power (*K*) of dextrose as 100, the reducing power of maltose is 62; these figures express the relative amounts of copper reduced by equal amounts of the two sugars. The amount of copper oxide actually obtained is calculated as the percentage of the amount which would be obtained if dextrose only were present. Let this = K. Then the percentage of maltose in the sugar mixture $= \frac{(100 - K)\,100}{100 - 62} = \frac{100 - K}{0 \cdot 38}$. The percentage of dextrose is then found by difference.

The optical activity, $[\alpha]_D$ is calculated by multiplying the observed angular rotation with sodium light by 100, and dividing by the concentration of the solution in grams per 100 c.c. and by the length of the tube in decimetres. For dextrose, $[\alpha]_D = + 52 \cdot 7°$, and for maltose, $[\alpha]_D = + 138°$. The percentages of the two sugars may then be calculated on the same lines as just described in the case of the reducing power.

It is not possible to estimate maltose in presence of dextrose by measuring the change in optical rotation and reducing power after hydrolysis by acid, as is done in the case of sucrose, for some of the dextrose is destroyed by the acid under the conditions necessary to secure the hydrolysis of maltose. Davis and Daish (footnote, p. 372) employ the biological method indicated on p. 372. Portions of 50 c.c. of the solution are fermented at 25° for three to four weeks with fresh pure cultures of different yeasts, and the changes in optical activity and reducing power produced by the fermentations are determined. The difference found between the average reducing power after fermentation with baker's yeast, and the average reducing power found after fermenting with the yeasts which do not attack maltose, is due to the maltose present. For details, the paper referred to may be consulted.

(C) *Glucose or Starch Syrup.*

This is the product of the action of dilute acid on starch or starch containing material. It is largely used as a substitute for sucrose in confectionery, and occasionally for adulterating cane sugar, syrup, honey, etc. The solid *grape sugar* is produced by more complete hydrolysis and consists largely of dextrose; this is also used in confectionery and in brewing. Starch syrup is a complicated mixture, varying in composition according to the extent to which the process of hydrolysis has been carried; further, many of the constituents, notably the dextrins, malto-dextrins and the unfermentable matter (gallisin) produced by the action of acid on dextrose, are substances of which little is known from the chemical side. Wesener and Teller[1] give the composition of commercial glucoses as follows: Water, 15 to 20 per cent.; proteins, about 0·06 per cent.; mineral matter, traces. The carbohydrates consist of dextrins, maltose and dextrose, of which the two last mentioned are fermentable. In two samples 11·7 and 17·2 per cent. of dextrose, and 22·9 and 16·4 per cent. of maltose, were found, respectively.

If the exact analysis of starch syrup is difficult, its estimation as an adulterant in presence of other carbohydrate products is still more difficult. Sometimes the reducing sugar is calculated as dextrose, which, in view of the above figures, is obviously incorrect. Unless, however, a very lengthy and detailed examination is made, the results obtained will only be approximately correct.

Water may be estimated by drying a few grams in a flat dish in the water oven, and weighing the residue. *Ash* may be determined in the usual manner on the dried residue, and *Proteins* by the Kjeldahl-Gunning method (p. 26). Deducting the ash from the total solids found in the water determination, the *total organic matter*, consisting almost exclusively of carbohydrates, will be found. The total carbohydrates may also be found with sufficient accuracy by taking the specific gravity of a 10 per cent. solution of the syrup and applying the method of calculation explained on p. 366.

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[1] *J.I.E.C.*, 1916, 8, 1009.

Dextrin, Maltose and Dextrose.—Assuming the organic matter to consist of these three carbohydrates, their percentages may be obtained approximately by the following method: Thirty c.c. of a 30 per cent. solution of the syrup are poured into 300 c.c. of approximately 95 per cent. alcohol (strong methylated spirit free from paraffin will serve) with stirring. On standing, the precipitated dextrin will collect as gummy matter on the sides and bottom of the vessel. If necessary, more alcohol may be added to obtain a clear separation. The alcoholic solution of the sugars is poured off, and the dextrin is washed with alcohol, dried to remove alcohol, and dissolved in water. The amount of dextrin can be found from the specific gravity of the solution, as explained on p. 366, using the factor 3·95. A known amount of the solution may also be evaporated, and the residue determined after drying to constant weight in the water oven. The dextrin may also be estimated polarimetrically in the solution, the specific rotation $[\alpha]_D$ being +200°. Sometimes the solution may be too dark for measurement by sodium light. The liability of dextrin to be absorbed, if the solution is cleared by lead acetate, should be borne in mind (see p. 313). The solution of dextrin should not reduce Fehling's solution.

Knowing the percentage of dextrin, the dextrose and maltose may be estimated by difference, and their relative proportions determined by the reducing power as described on p. 378, dextrin being non-reducing.

The results obtained by the above methods may be checked by determining the specific rotation S of the carbohydrates in a solution of the syrup in which the total carbohydrates T is known. If g = the percentage of dextrose, m that of maltose and d that of dextrin, then

$$S = \frac{52 \cdot 7\,g + 200\,d + 138\,m}{T}.$$

Owing, however, to experimental errors and our incomplete knowledge of the composition of starch syrup, this relationship will only be found approximately correct.

Grape Sugar.—This product has been mentioned above as a product of the action of acid on starch (see p. 380). The products of complete hydrolysis which have been recrystallised approximate to pure dextrose in composition, present fewer analytical difficulties than starch syrup, though in accurate work the reducing unfermentable product of the action of the acid on dextrose, known as "gallisin," must be reckoned with. Leach gives the following figures as representing the variations in composition of commercial grape sugar: Dextrin, 0—9·1 per cent.; maltose, 0—1·8 per cent.; dextrose, 72—99·4 per cent.; ash, 0·3—0·75 per cent.; water, 0·6—17·5 per cent.

SUCROSE.

This, the commonest of the sugars of commerce, is found in many plants, but is almost exclusively obtained from the sugar cane and sugar beet. The sugar cane juice is obtained by a process of crushing. The beet is sliced and extracted with water, the process being arranged progressively so that fresh material is treated with water already containing extract, while nearly exhausted material is treated with fresh water. Proteins and organic acids are removed from the juice by heating and nearly neutralising with milk of lime, and the raw sugar is obtained by crystallisation and centrifuging. The refining process consists in heating the raw sugar solution with such substances as lime, clay blood, animal charcoal, etc., and crystallisation. The mother liquors obtained from these processes are known as molasses. Cane molasses consists chiefly of cane sugar, invert sugar and water, but beet molasses, which is unfit for human food, is a much more complex mixture.

The best granulated, loaf or castor sugars of commerce are generally 99·8 per cent. pure sucrose. Inferior grades may contain up to about 5 per cent. or more of water, 2 per cent. (or more, if very poor quality) of reducing sugar as dextrose, and up to about 2 per cent. of ash. Small traces of ultramarine or blue aniline dyes are added to counteract the yellow colour so as to give a white appearance, while some sugars are coloured yellow so as to simulate the original Demerara sugar.

Water may be determined by heating a few grams of the powdered sugar at 105° till constant in weight. *Ash* may be determined by moistening 5 grams of the sugar with pure concentrated sulphuric acid and igniting till the residue is white. One-tenth of the sulphated ash is deducted to obtain the true ash. Dyes are detected as described in the following chapter, p. 441. *Reducing Sugar* is determined as dextrose by the methods given on pp. 373 to 376.

ESTIMATION OF SUCROSE (1) IN ABSENCE OF OTHER SUGARS OR IN PRESENCE OF INVERT SUGAR.

The *direct polarimetric method*, using polarimeters with special "sugar scales," has been referred to on p. 370. This method is, of course, only of use in the absence of sugars other than sucrose. If the reading is taken in angular degrees, the percentage of sucrose may be calculated from its specific rotation, $[\alpha]_D = 66{\cdot}5°$, using a 10 per cent. solution at 20° C.

Solutions of *dark coloured sugars, treacles, syrups or molasses* must be clarified before they can be observed in the polarimeter. Basic lead acetate is the most commonly used clearing agent.

Walker[1] has devised the following modified method for clearing solutions of molasses so as to obviate the error due to the volume occupied by the precipitated lead when the sugar solution is made up to a definite volume. A double normal weight of the sample, 52 grams (26 grams is the normal weight giving in 100 c.c. the percentage of sucrose on the scale of more recent type of Ventzke saccharimeter), is dissolved in water and made up to 300 c.c. The solution is transferred to a larger flask, and clarified with 15 to 20 grams of dry basic lead acetate and a few grains of sand, and filtered through a dry filter. The volume of the solution is increased through the solution of the basic lead acetate, and the process has been so devised that the error due to this cause is practically balanced by the error due to the volume of the lead phosphate precipitated in the next operation. To 75 c.c. of the filtrate in a 100 c.c. flask are added 20 c.c. of a solution containing 100 grams of phosphoric acid per litre, and the liquid is made up to 100 c.c. and filtered. If necessary, the solution may be further decolorised by the addition of 0·5 gram of zinc dust just before filtration. The polarimetric reading is taken in a 400 mm. tube; it will be noticed that the dilution is now equivalent to a normal weight in 100 c.c. If the reading is taken in angular degrees, multiplication by the factor 2·8889 will give Ventzke degrees for the above normal weight, which correspond to the percentage of sucrose. For the older instruments of the same type, the normal weight is 26·068 grams, and the corresponding factor for converting angular degrees is 2·8835.

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[1] *J.I.E.C.*, 1918, 10, 198.

The method of direct polarimetric measurement is only reliable when applied to pure or fairly pure products. If any notable amount of invert sugar is present, as will be the case with the cruder products, *Clerget's process* of determining the *change in optical rotation after inversion* should be applied. Walker continues the process as follows: Another portion of 75 c.c. of the filtrate from the basic lead acetate is treated in a 100 c.c. flask with 2 c.c. of hydrochloric acid (concentrated acid diluted with an equal volume of water) to neutralise the alkalinity. The mixture is heated in a water bath to 65°—70° treated with 10 c.c. of the same hydrochloric acid as just used, taken out of the water bath and allowed to stand for 15 minutes, after which it is cooled, made up to 100 c.c., treated with 0·5 gram of zinc dust and filtered for polarimetric reading. The percentage of sucrose, S, is given by the formula $S = \frac{(D - I)\,100}{142{\cdot}1 - 0{\cdot}5t}$ where D = the direct sugar scale reading, I = the reading after polarisation, and t = the temperature of the solutions when both readings were taken. For the reason stated below, it is advisable to take the readings in jacketed tubes through which water is kept circulating at a definite temperature.

The Clerget formula is based on the fact that at 0° a sucrose solution, which gives a reading of 100 divisions to the right, gives, after inversion, a reading of 144 divisions to the left. According to the International Commission methods, when the readings are taken at 20° with a normal weight of 26 grams, the constant 142·66 is used in the above formula. Walker uses the constant 142·1. The specific rotations of all sugars vary to some extent with the temperature but that of lævulose falls especially rapidly with rise of temperature. Hence the temperature correction in the formula, and the particular necessity for attention to this point when dealing with solutions containing lævulose.

If readings are taken in angular degrees, conversion into sugar scale degrees may be made as described above, and the values thus found substituted in the Clerget formula. Different types of instrument require different normal weights, the factors connecting scale degrees with angular degrees varying correspondingly.

Many methods of clarification have been proposed; most of them involve the use of lead acetate, but do not correct for precipitate volume as the one described. Animal charcoal clarifies, but it should be avoided as it adsorbs sugars.

The usual method of inverting by acid is to add 10 per cent. by volume of hydrochloric acid, specific gravity 1·188, to the sugar solution, to heat to 69° in two to two and a half minutes, and to maintain the mixture at this temperature for seven to seven and a half minutes, the total time of heating being ten minutes. In all cases the solution should be freed from lead before inversion.

Inversion by hydrochloric acid for the estimation of sucrose is only admissible when the mixture contains no other disaccharide than sucrose and no polysaccharides, such as dextrin or starch. The method is, however, applicable to mixtures of sucrose and invert sugar, such as occur in *raw sugar, cane molasses, treacle or golden syrup*. The percentage of cane sugar being known, the percentage of invert sugar can be calculated from the rotation of the solution before inversion, the specific rotation $[\alpha]_D$ of sucrose being $+66{\cdot}5°$, and that of invert sugar being $-20{\cdot}5°$ at 15°.

Sucrose may also be estimated in presence of invert sugar by the *change in cupric reducing power after inversion*, by the methods described on pp. 373 to 376. If the volumetric method of Ling and Rendle is used, a standard solution of invert sugar may be prepared by inverting a solution containing a known percentage of pure sucrose. The reduction, if any, before inversion will be due to invert sugar, and the increase in reduction after inversion will be due to invert sugar produced from the sucrose. The weight of the latter, multiplied by 0·95, will give the weight of cane sugar.

ESTIMATION OF SUCROSE (2) IN PRESENCE OF STARCH SYRUP.

Such cases occur when *molasses or golden syrup are adulterated with starch syrup*. In the first place, this problem is rendered difficult owing to the uncertain composition of starch syrup. Leach [1] gives the following formula for the calculation of the percentage of glucose or starch syrup, provided that the amount of invert sugar is small. It is stated to give fairly satisfactory results with golden syrup and molasses, but better results with maple syrup, which contains less invert sugar. The formula is an empirical one, being based on the fact that the maximum rotation on the sugar scale (Ventzke), shown by the glucose syrup commonly added, is 175. Under the circumstances it becomes necessary to resort to the use of a formula of this kind unless a prolonged investigation is made.

If A = the direct polarimetric reading, and S = the percentage of sucrose, then the percentage of glucose syrup, $G = \frac{(A - S)\,100}{175}$.

Here, again, conversion from angular degrees into sugar scale degrees may be made for the purposes of the formula, as described in the preceding section. The sucrose is determined in the usual way by inversion. It must be noted that the results for sucrose cannot fail to be affected to some extent owing to the simultaneous action of the acid on the dextrin and maltose, and change in optical activity due to these causes. In such cases it is better to employ the enzyme invertase for inversion.

Inversion by Invertase.—The enzyme may be prepared by the method given in Allen's "Commercial Organic Analysis" by E. F. Armstrong. Brewer's yeast is allowed to liquefy by keeping it for several days at 37°, and then filtered. The enzyme is partially purified by precipitation with alcohol and redissolving in a minimum of water. The extract, which is free from maltase, keeps well in closed vessels in the dark, in presence of a little toluene, which is used as an antiseptic.

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[1] U.S. Dept. of Agric., Bureau of Chem., Bull. 65.

The solution may be cleared, if necessary, and freed from lead by the method given on p. 384. 75 c.c. of the filtrate are measured into a 100 c.c. measuring flask, neutralised and rendered faintly acid with acetic acid. A few drops of toluene and a few c.c. of the invertase extract are added, and the mixture is kept at about 50° for five hours. The volume is then completed to 100 c.c. below the toluene layer, and the polarimetric reading is taken. As invertase acts only on sucrose, the change in rotation will be due to this sugar only.

Qualitative Detection of Starch Syrup.—The presence of starch syrup is indicated by a high initial dextrorotation while, if much starch syrup is present, the liquid may fail to become lævorotatory after inversion. *Dextrin* may be precipitated by alcohol and estimated as described on p. 381. Starch syrup may also be indicated by the presence of notable amounts of calcium when the solution is tested with ammonium oxalate.

ESTIMATION OF THE CONSTITUENTS IN A MIXTURE OF SUCROSE AND STARCH SYRUPS.

The total carbohydrates, and incidentally the *water* and *ash,* are determined as described on p. 378. The sucrose having been determined by inversion with invertase, as described above, the rotation due to the invert sugar and the starch syrup may be calculated. *Invert sugar* may be approximately estimated by estimating the lævulose by the change in rotation on rise of temperature and assuming the presence of an equal amount of dextrose. This may be done by observing the specific rotation at two temperatures fairly widely apart, the decrease in the specific rotation of lævulose being 0·6385 per degree C. rise. The percentage of lævulose, $L = \frac{100\,D}{0{\cdot}6385\,t.l}$, where D = the difference in polarimetric readings in angular degrees, t = the difference between the two temperatures, and l = the length of the tube in decimetres. The method is not strictly accurate, for other sugars also show changes in optical activity with change in temperature, though not to so marked an extent as lævulose. *Dextrin* may be estimated by the alcohol precipitation method. The sucrose, dextrin and invert sugar having been determined, the rotation due to the *maltose and the dextrose belonging to the starch syrup* may be calculated; this, together with the combined weights of these constituents obtained by difference, will enable the relative proportions of dextrose and maltose to be calculated (for invert sugar, $[\alpha]_D = -20{\cdot}5$ at 15°).

Such methods of calculation have their limitations, for the last results bear the accumulated errors of the previous determinations. A check can be obtained by the cupric reducing power, which will be due to the invert sugar, dextrose and maltose, but even then, the gallisin (see p. 380) is left out of account. Again, the composition of starch syrup may be even more complex than has been assumed.

A step further would be to destroy the fermentable carbohydrates by means of ordinary yeast (cf. p. 371), leaving the dextrin (non-reducing) and the gallisin (reducing) to be determined in the fermented liquid. The estimation of maltose by differential fermentation has been touched upon above (see p. 372).

ESTIMATION OF SUCROSE (3) IN PRESENCE OF LACTOSE.

Such a mixture occurs in *sweetened condensed milk.* The estimation of lactose in ordinary milk by the cupric reduction method has been described on p. 276. The Gayaux test for sucrose in milk is given on p. 293. Elsdon[1] has reviewed the various tests for sucrose in presence of lactose, and gives preference to the Gayaux test in a modified form.

Polarimetric Method for estimating Lactose and Sucrose in Milk.—The method to be described may be used for the determination of lactose in ordinary milk; if sucrose is present, it may be estimated by inversion on the same lines as described under the preceding headings. Condensed milk will be assumed to have been diluted to about the same specific gravity as ordinary milk.

To obtain a clear whey for polarimetric reading, Wiley's acid mercuric nitrate is generally used. This is prepared by dissolving mercury in twice its weight of nitric acid of specific gravity 1·42 and diluting the solution with an equal volume of water. In order to correct for the volume of the precipitated fat and proteins, and the specific gravity of the solution, Richmond and Bosely's method[2] may conveniently be used. The scale readings in angular degrees can be read as percentages of lactose (in the absence of sucrose) if to 100 c.c. of milk are added, (*a*) a volume of water in c.c. = one-tenth of the degree of specific gravity (see p. 258); (*b*) a volume of water in c.c. = the fat percentage multiplied by 1·11; (*c*) a volume of water to reduce scale readings to percentages of lactose; with a 200 mm. tube, and reading in angular degrees, the volume is 10·85 c.c.; (*d*) 8 c.c. of the acid mercuric nitrate solution. The mixture is shaken and filtered through a dry filter and the polarimetric reading of the filtrate is taken. Dilutions necessary when using other tube lengths and other types of polarimeter are given in the paper referred to, and in Richmond's "Dairy Chemistry."

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[1] *Analyst*, 1918, 292. [2] *Analyst*, 1897, 98.

If sucrose is present, the reading will be due to the combined effect of the lactose and sucrose. Inversion for the purpose of estimating sucrose is best effected by the use of invertase, the preparation of which is described on p. 388. Yeast may also be used. Richmond gives the following directions :—

25 c.c. of the filtrate from the acid mercuric nitrate treatment just described are placed in a flask, a drop or two of phenol phthalein added, and dilute caustic soda solution is run in till the liquid is neutral. The liquid is filtered into a 50 c.c. flask, and the precipitate washed with water until the filtrate measures about 45 c.c. 2 or 3 c.c. of invertase extract or a gram of yeast, previously rubbed up with a little water, together with a drop of acetic acid and a few drops of toluene are added, and the whole is made up to 50 c.c. The flask is corked and allowed to stand at 55° for five hours. A little alumina cream is added and the whole made up to 55 c.c., filtered and examined in the polarimeter. The alumina cream is made by adding ammonia to a saturated solution of alum till slightly alkaline, and then more alum solution till the mixture is faintly acid; this clears the turbidity produced by the yeast. It will be noted that the toluene (as well as the high temperature employed) hinders any fermenting action on the part of the yeast, but not the action of the enzyme. Volumes

are taken beneath the toluene layer. The reading is multiplied by 2·2 to allow for dilution. The Clerget formula $Rs = \frac{100\,(R - I)}{142{\cdot}66 - 0{\cdot}5t}$ gives the rotation Rs due to the sucrose, where R is the original rotation, I the rotation after inversion multiplied by 2·2, and t the temperature at which both readings have been taken. If the dilution has been standardised by the above method so as to give direct readings of the percentage of lactose, then the percentage of sucrose may be calculated by multiplying Rs by 66·5, the specific rotation of sucrose, and dividing by 52·5, the specific rotation of lactose.

Inversion may also be effected by boiling with 2 per cent. citric acid which leaves the lactose unaffected (see p. 371). The proteins and fat of milk are precipitated by dilute citric acid, but difficulty is often experienced in preparing clear solutions by this method.

If *invert sugar or glucose* should be present as well, the apparent figure found for lactose will be abnormal in proportion to the other milk solids. This will also show itself in a discrepancy between the total solids as estimated and as calculated from the sum of the fat, proteins, ash, sucrose and apparent lactose. (See p. 283 and p. 293.) In such a case it will be necessary to ferment with ordinary yeast, which will destroy the sucrose and invert sugar, leaving the lactose which can then be estimated. The fermentation method is described in the following section, together with the citric acid method of inversion.

Carbohydrates in Infants' Foods.—The methods to be described were used by Baker (footnote 2, p. 311) in the examination of the cold water extract from infants' foods (see p. 359).

For the estimation of *dissolved carbohydrates*, see p. 366. *Soluble proteins*, if present, can be determined in the residue obtained by evaporating a known volume of the solution, by the Kjeldahl method (see p. 26).

Reducing Sugars as Dextrose other than Lactose.—These were determined by the volumetric method of Ling and Rendle given on p. 373. If lactose was present, the reduction due to it was found as described below, and allowed for. The sugars in question were mostly dextrose and lævulose, but as they were only present in small amounts no great error was committed in returning them as dextrose.

Cane Sugar.—This was determined by the increase in cupric reduction after boiling with citric acid. 2 per cent. of citric acid should be used, and the solution boiled for at least 30 minutes. The increased reduction is due to invert sugar, and the amount of invert sugar thus found multiplied by 0·95 gives the sucrose. If more than 2 per cent. of sucrose was found, the invertase method was used (see p. 388).

Lactose.—The solution was boiled with 2 per cent. of citric acid to invert any sucrose and thus to facilitate its fermentation. It was then exactly neutralised, cooled and treated with a little cold water extract of a diastatic malt (see p. 354), which secures the decomposition of malto dextrins. (The addition of diastase would be unnecessary in dealing with a milk product containing only sucrose and invert sugar.) For 2 to 3 per cent. of mixed sugars, half a gram of freshly washed brewer's yeast was added per 100 c.c., together with a little yeast water if the solution was non-nitrogenous. The flask was closed with a cotton wool plug and kept for seventy-two hours at 27°. After clearing with a little alumina cream (see p. 392), the solution was filtered, boiled, made up to a definite volume and titrated with Fehling's solution. It was found in control experiments that the results were usually about 5 per cent. too low. On the other hand, the malto dextrins were found to leave a small unfermented residue which reduces Fehling's solution, the error amounting to from 3 or 4 per cent. of the material fermented. The error would be proportional to the proportion of hydrolysed starch products present.

Hydrolysed Starch Products.—These were determined by deducting from the total carbohydrates the other soluble bodies which had been determined separately.

PART II.

ALCOHOL AND ALCOHOLIC FERMENTATION.

Alcoholic fermentation is usually understood to consist essentially in the decomposition of certain monosaccharides into carbon dioxide and alcohol, through the agency of micro-organisms, notably yeasts. The question as to whether any particular disaccharide or polysaccharide will be fermented in this way, depends, first, on whether the organism contains an enzyme capable of hydrolysing it to monosaccharides, and, second, whether it contains an enzyme capable of fermenting the resulting monosaccharide. In the case of ordinary English brewer's yeast, *Saccharomyces cerevisiae*, both conditions are fulfilled with respect to sucrose and maltose; but, as mentioned on p. 372, certain yeasts are known which contain no maltase, and consequently do not fulfil the first condition with respect to maltose, although they will ferment dextrose. Again, starch is not directly fermentable by ordinary yeast, as this contains no diastase, but certain moulds, *Amylomyces Rouxii* and *Mucors* B. and C. contain both diastase and zymase, and are therefore able to convert starch into alcohol without previous saccharification by diastase or mineral acids. This type of fermentation is employed in the amylo process, which is used on the continent, but has not been permitted in England for the somewhat inadequate reason that it does not lend itself to control by the existing excise methods. Starch is saccharified for the fermentation by yeast, either by the action of dilute mineral acid which converts it into dextrose (see p. 373), or by the action of the diastase of malt which converts it into dextrin and maltose. In the manufacture of beer and some spirits, diastatic action precedes alcoholic fermentation; the alcohol in wine is produced by the direct fermentation of the sugar of the grape; brandy is the distillation product of fermented grape juice, whisky is generally derived from barley, and rum is the fermentation product of molasses or sugar cane juice. Much potable spirit is, however, made by flavouring and diluting alcohol prepared for the purpose.

In addition to the many industrial uses of alcohol, *e.g.* as a solvent for many substances and in chemical synthesis, the question as to its possible use as a source of power as a substitute for petrol has come to the front in recent years.

Alcohol is obtainable from a very large variety of vegetable substances. Among the starchy raw materials which furnish the bulk of the alcohol produced, may be mentioned potato, maize, grain and waste matter from the manufacture of starch. Sugar containing raw materials are beet, sugar cane and beet molasses, and various fruits and flowers ; in this connection, mention may be made of the Indian Mowra flowers (*Bassia Latifolia*), which are at present treated for the production of alcohol by primitive methods. In Sweden and Norway, alcohol is obtained from the sugar contained in the sulphite lye, arising through the hydrolysis of part of the cellulose during the manufacture of wood pulp. In a similar way, alcohol can be produced from peat.

As might be expected of the product of a vital process from naturally occurring substances, fermentation alcohol contains various bye products ; its value depends on its relative freedom from such products.

Estimation of Alcohol.—If carbon dioxide is present, this is first removed by repeatedly pouring the liquid from one vessel to another, or by filtration, and the sample is made up to 100 c.c., or, if necessary, to a larger definite volume, with distilled water. 100 c.c. are then distilled from a flask of convenient size connected with a straight tube condenser, until 80 c.c. of distillate have been collected. The distillate is made up to 100 c.c. with distilled water, and its specific gravity determined at 60° F. by means of the pyknometer or specific gravity bottle.[1] Reference to the accompanying tables will show the percentage of alcohol in the distillate. In order to calculate the percentage of absolute alcohol in the sample before distillation, it will be necessary to know either its specific gravity and volume or its weight, and likewise,

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[1] For a description of the exact determination of specific gravities of liquids, see Allen's "Commercial Analysis," Vol. I., pp. 5 and 6, 1909 edition.

TABLES (ABRIDGED) ADOPTED BY THE AMERICAN ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.

PERCENTAGE OF ALCOHOL AND SPECIFIC GRAVITY.

FOR MIXTURES OF ALCOHOL AND WATER.

(Recalculated from the determinations of Gilpin, Drinkwater and Squibb, by E. Richards.)

Specific Gravity at $\frac{60^\circ}{60^\circ}$ F.	Per cent. Alcohol by Volume.	Per cent. Alcohol by Weight.	Specific Gravity at $\frac{60^\circ}{60^\circ}$ F.	Per cent. Alcohol by Volume.	Per cent. Alcohol by Weight.	Specific Gravity at $\frac{60^\circ}{60^\circ}$ F.	Per cent. Alcohol by Volume.	Per cent. Alcohol by Weight.	Specific Gravity at $\frac{60^\circ}{60^\circ}$ F.	Per cent. Alcohol by Volume.	Per cent Alcohol by Weight.
1·00000	0·00	0·00	0·98381	12·50	10·08	0·97097	25·00	20·43	0·95560	37·50	31·14
0·99923	0·50	0·40	0·98326	13·00	10·49	0·97044	25·50	20·85	0·95487	38·00	31·58
0·99849	1·00	0·79	0·98273	13·50	10·90	0·96991	26·00	21·27	0·95413	38·50	32·03
0·99775	1·50	1·19	0·98219	14·00	11·31	0·96937	26·50	21·69	0·95338	39·00	32·46
0·99701	2·00	1·59	0·98167	14·50	11·72	0·96883	27·00	22·11	0·95262	39·50	32·90
0·99629	2·50	1·99	0·98114	15·00	12·13	0·96828	27·50	22·54	0·95185	40·00	33·35
0·99557	3·00	2·39	0·98063	15·50	12·54	0·96772	28·00	22·96	0·95107	40·50	33·79
0·99487	3·50	2·80	0·98011	16·00	12·95	0·96715	28·50	23·38	0·95028	41·00	34·24
0·99417	4·00	3·20	0·97960	16·50	13·37	0·96658	29·00	23·81	0·94948	41·50	34·68
0·99349	4·50	3·60	0·97909	17·00	13·78	0·96600	29·50	24·23	0·94868	42·00	35·13
0·99281	5·00	4·00	0·97859	17·50	14·19	0·96541	30·00	24·66	0·94786	42·50	35·58
0·99215	5·50	4·40	0·97808	18·00	14·60	0·96181	30·50	25·08	0·94704	43·00	36·03
0·99149	6·00	4·80	0·97758	18·50	15·02	0·96421	31·00	25·51	0·94620	43·50	36·48
0·99085	6·50	5·21	0·97708	19·00	15·34	0·96360	31·50	25·94	0·94536	44·00	36·93
0·99021	7·00	5·61	0·97658	19·50	15·84	0·96298	32·00	26·37	0·94450	44·50	37·39
0·98959	7·50	6·02	0·97608	20·00	16·26	0·96235	32·50	26·80	0·94364	45·00	37·84
0·98897	8·00	6·42	0·97558	20·50	16·67	0·96172	33·00	27·23	0·94276	45·50	38·30
0·98837	8·50	6·83	0·97507	21·00	17·09	0·96108	33·50	27·66	0·94188	46·00	38·75
0·98777	9·00	7·23	0·97457	21·50	17·51	0·96043	34·00	28·09	0·94098	46·50	39·21
0·98719	9·50	7·64	0·97406	22·00	17·92	0·95977	34·50	28·52	0·94008	47·00	39·67
0·98660	10·00	8·04	0·97355	22·50	18·34	0·95910	35·00	28·96	0·93916	47·50	40·13
0·98603	10·50	8·45	0·97304	23·00	18·76	0·95842	35·50	29·39	0·93824	48·00	40·60
0·98546	11·00	8·86	0·97253	23·50	19·17	0·95773	36·00	29·83	0·93730	48·50	41·06
0·98491	11·50	9·27	0·97201	24·00	19·59	0·95703	36·50	30·26	0·93636	49·00	41·52
0·98435	12·00	9·67	0·97149	24·50	20·01	0·95632	37·00	30·70	0·93540	49·50	41·99

also, the specific gravity and volume or the weight of the distillate.

Then

$$\frac{\text{Sp. gr. of distillate} \times \text{vol. of dist. in c.c.} \times \text{\% of alcohol in dist.}}{\text{Sp. gr. of sample} \times \text{vol. of sample in c.c.}}$$

= Percentage of absolute alcohol by weight contained in the sample, or

$$\frac{\text{Weight of dist.} \times \text{\% of alcohol in dist.}}{\text{Weight of sample taken}}$$

= Percentage of absolute alcohol by weight contained in the sample. 100 parts of alcohol correspond to 169·2 parts of starch.

The above method for estimating alcohol is commonly applied to beers, wines and spirits. It is used largely for the determination of the "original gravity" of fermented worts, *i.e.*, the specific gravity of the wort before fermentation, for excise purposes. The "original gravity apparatus" consists essentially of a distilling flask connected to a vertically placed spiral condenser, of certain dimensions; by determining the specific gravities of the still residue and the distillate, both diluted to the original volume of the sample, it is possible to calculate the number of "degrees of specific gravity" lost by the wort during fermentation.

In determining the alcohol in beer, 100 c.c. of the sample may be distilled till 80 c.c. of distillate have been collected. Spirits containing about 50 per cent. or more of alcohol should be diluted before distilling; thus, 50 c.c. of the sample may be diluted with 100 c.c. of distilled water, and distilled until the distillate measures 100 c.c.

The further examination of spirits includes the determination of minute amounts of esters, aldehydes, higher alcohols, fusel oil, furfural; an acidity, volatile and fixed. Wines are also examined for extract, sugar, ash, fixed and volatile acids, etc., and beers for extract, ash, acidity, etc. The examination for artificial colouring matters and preservatives is dealt with in the next chapter. For further information on the examination of these products, the works on Food Analysis mentioned at the end of this chapter, and Chapters VII. and IX., may be consulted.

IMPURITIES OF RAW SPIRIT.

The chief impurities are fusel oil, *i.e.* the normal and iso propyl, butyl and amyl alcohols, acetaldehyde furfural, acids and ethyl esters. Certain substances giving rise to characteristic tastes and aromas, but which are very difficult to identify chemically, may also be present; their nature depends on the raw material and the method of manufacture; mostly they are unpleasant, especially those in alcohol made from beet molasses, peat and wood. Artificial flavouring matters are largely added to potable spirits to simulate the characteristic flavours which originally arose naturally from certain raw materials treated by certain methods.

The chief methods for the purification of spirit are fractional distillation and filtration over charcoal. In the rectification by modern methods, the fusel oil and furfural are removed, and the production of esters from the alcohol and acids during this process is avoided by the addition of alkali. Aldehyde can be removed by warming the 40 per cent. spirit to about 50° to 60° and passing a current of air over the surface.

In the valuation of raw or rectified spirit, the *taste and smell* are important, and proper judgment on these points can only be given by experts on the subject. It is often possible to trace defects in raw materials or methods of manufacture to their origin by the taste of the product. The same also applies to such materials as oils and fats, milk and dairy products (see p. 111).

In addition to having a taste and smell which may be described as neutral, a good rectified spirit should be *colourless*, and give a *clear colourless mixture on the addition of water.*

Aldehydes.—To 10 c.c. of the spirit is added 1 c.c. of a freshly made 10 per cent. solution of the purest metaphenylene diamine hydrochloride in distilled water. After ten minutes the colour is observed, and compared with the colours produced under exactly similar conditions with solutions of known aldehyde content which have been prepared from pure aldehyde ammonia.

Good rectified spirit should show no aldehyde or only minute traces; raw spirit may contain up to about 1·5 grams of aldehyde as acetaldehyde per litre.

Furfural.—This may be estimated colorimetrically by the reaction given on p. 332. To 10 c.c. of the spirit are added 1 c.c. of pure acetic acid (see p. 127) and 1 c.c. of freshly distilled pure aniline. The colour is observed after five minutes and compared with the colours produced by solutions of furfural of known strength, under exactly similar conditions.

Good rectified and raw spirits should show no furfural or only minute traces.

Free Acid.—This may be determined by titration with twentieth normal sodium hydroxide solution, using phenol phthalein as indicator.

Good rectified spirit should only require at the most about 0·2 c.c. of alkali solution calculated as decinormal for neutralisation per 100 c.c. Raw spirit may require considerably larger amounts.

Fusel Oil.—Komarowsky's reaction for the detection of fusel oil is as follows: To 10 c.c. of the spirit are added 1 c.c. of a 1 per cent. solution of the purest salicylaldehyde in pure alcohol; 20 c.c. of pure sulphuric acid are then added carefully, and the whole is mixed; after twelve hours, the colour is observed.

Pure spirit gives only a bright yellow colour, while spirit containing up to about 1 or 2 per cent. of fusel oil gives a red to dark red colour. This test may also be made quantitative on the same lines as described for the preceding tests.

To carry out the above colour tests, pure chemicals are required, and it is advisable to make blank tests with alcohol of known purity.

Esters.—These are determined by boiling with a known volume of twentieth normal sodium hydroxide under conditions precluding the absorption of carbon dioxide from the air, and the amount of alkali used up in the saponification of the esters is determined by titration, allowance being made for the amount of alkali used up in neutralising the free acid. The esters are calculated as ethyl acetate. The process is comparable with the determination of the saponification or ester value of fats (see p. 131).

In *whisky* or *brandy* the above impurities are determined on the distilled sample, and calculated as parts per 100,000 of absolute alcohol present. The distillate on which the tests are made is adjusted to contain 50 per cent. of alcohol. It is necessary to use an efficient condenser as some of the products to be estimated are very volatile.

REFERENCES

Villavecchia and Molinari's works. See end of Chapter VII.

Allen's "Commercial Organic Analysis," Vol. I. See end of Chapter I.

"The Sugars and their Simple Derivatives," by J. E. Mackenzie. 1913. Gurney & Jackson, London.

"The Simple Carbohydrates and Glucosides," by E. F. Armstrong. 1919. Longmans, London.

"Handbook of Sugar Analysis," by Browne. 1912.

"Beet Sugar Manufacture," by Claassen, trans. by Hall and Woolfe. 1910. John Wiley, New York; Chapman & Hall, London.

"Sugar Analysis," by Weichmann. 1914.

"Technical Mycology," by Lafar. 1911. 2 vols. Gustav Fischer, Jena.

"Alcoholic Fermentation," by Harden. 1914. Longmans, Green, London.

"Micro-organisms and Fermentation," by Jörgensen, trans. by Davies. 1911. Chas. Griffin, London.

"Industrial Alcohol," by M'Intosh. 1907. Scott, Greenwood, London.

CHAPTER IX

PRESERVATIVES AND ARTIFICIAL COLOURING MATTERS IN FOODS

PART I.—PRESERVATIVES

INTRODUCTORY

ALL foodstuffs are liable to decomposition and decay through the agency of micro-organisms, and if they are to be kept for any length of time in a state fit for human consumption, it is sometimes necessary to adopt some means for their preservation, by which the micro-organisms which they contain may either be destroyed or temporarily rendered sufficiently inactive to prevent them from appreciably affecting the food.

The method of heat sterilisation and subsequent preservation in hermetically closed vessels, as practised in the canning industry, has for its object the destruction of the micro-organisms by heat (see Chapter VI.) and the protection of the food from further contamination until it reaches the consumer. Preservation by cold storage is based on the fact that micro-organisms become inactive at low temperatures, while the treatment of the food with wood smoke, salt, sugar or other substances has a similar effect, *i.e.*, the temporary checking of the activity of the micro-organisms, without, however, destroying them.

Heat sterilisation, if efficiently carried out, is naturally more thorough in its effects than the other methods just mentioned, though it has a real disadvantage when applied to many foodstuffs owing to the partial decomposition suffered by proteins and carbohydrates under the influence of heat. (See Chapter VI., the Heat Sterilisation and Pasteurising of Milk, also Condensed Milk.)

In cases when, for this and other reasons, the food cannot be completely sterilised by heat, recourse may also be had to cold storage and sometimes also the addition of preservatives. Cold storage and addition of preservatives may also be practised simultaneously. The merits and disadvantages of the latter method will be discussed below; it may, however, be pointed out here that, generally speaking, it would probably be in the best interest of the consumer if the method of cold storage were employed in preference to the addition of preservatives, wherever possible.

In the present chapter we are concerned with the method of preserving by the addition of certain chemicals with the object of inhibiting the growth and development of micro-organisms in the food; among the substances which have been used in this capacity, the following may be mentioned: Common salt, boric acid and borax, sodium fluoride, sulphites and sulphurous acid, benzoic acid and benzoates, salicylic acid and salicylates, formaldehyde, β naphthol, abrastol or asaprol (the calcium salt of β naphthol sulphonic acid), hydrogen peroxide, saccharin, formic acid and formates, nitrates and nitrites. As regards the advisability of permitting such substances to be added to foods, two main questions come into consideration:—

Firstly, the question as to whether the substance added is injurious to health, even in minute proportions, or has an undesirable effect on the food with which it comes into contact, and if not, the maximum proportion in which it can be added without prejudice to the purchaser. While the use of such a preservative as common salt has long been recognised and sanctioned in all countries, considerable diversity of opinion still exists as to the advisability of permitting the use of certain preservatives, such as borax, boric acid or benzoic acid in small quantities; other preservatives, such as sodium fluoride and formaldehyde, are generally looked on as distinctly injurious to health.

Secondly, there is the question as to whether, with proper organisation, care and cleanliness, it is possible to supply a given article of food to the consumer in a fresh and pure state, without the addition of preservatives. If this be the case, then the use of preservatives must obviously be looked on as decidedly objectionable, as it may often be practised in order to temporarily mask uncleanliness in treatment. This point will be further discussed below, in dealing with the subject of preservatives in milk.

The law of the United Kingdom does not definitely prohibit the use of any given substance as a preservative for foods, nor does it actually set any definite limit as to the proportion in which any given preservative may be used.

Section 3 of the Sale of Foods and Drugs Act of 1875 provides that "No person shall mix, colour, stain, or powder . . . any article of food with any ingredient or material so as to render the article injurious to health, . . . under a penalty in each case not exceeding fifty pounds for the first offence; every offence, after conviction for a first offence, shall be a misdemeanour, for which the person, on conviction, shall be imprisoned for a period not exceeding six months with hard labour."

Very few prosecutions have been instituted under this section, and practically all prosecutions are instituted under section 6 of the same Act, which is to the following effect:—

"No persons shall sell to the prejudice of the purchaser any article of food or any drug which is not of the nature, substance, and quality of the article demanded by such purchaser, under a penalty of twenty pounds. . . ." Exceptions are made in the case of foreign matter not injurious to health, necessarily or unavoidably added in the preparation of the food or drug, and not with the object of fraudulently increasing its bulk or weight, or concealing its inferior quality; also in the case of proprietary or patent medicines, supplied in a state required by the specification of the patent.

The purchaser is not held to be prejudiced if notice was given him at the time of the sale that the article sold was not of the nature, substance and quality of the article demanded; on the other hand, in order to show that the article was sold to the prejudice of the purchaser, it is not necessary to show that he has sustained actual prejudice or damage.

Questions as to whether the purchaser shall be held to have been prejudiced by the addition of material injurious to health to the article supplied, or otherwise, are usually decided with reference to the opinions and recommendations of the Medical Officers of Health and other experts. No further legislation has resulted from such recommendations except the Public Health (Milk and Cream) Regulations of 1912, referred to below under the heading of Preservatives in Milk, Cream, Butter and Margarine, and the Ministry of Food order prohibiting the addition of colouring matter or water to milk, not sold for consumption on the premises of the seller. This matter will be more fully dealt with below, under the various headings.

In the United States the law forbids the sale of food containing poisonous or deleterious substances which may render the food injurious to health, but does not definitely prohibit or limit the use of any given preservative. Some preservatives, such as boric and salicylic acids, and formaldehyde, are, however, held to be injurious to health, and prosecutions have been successfully maintained against them; the matter is at present under investigation.

In France, Germany, Austria-Hungary and Holland, the law is, generally speaking, more explicit and stringent with regard to the use of preservatives; either certain preservatives may be totally prohibited, or the use of preservatives in certain foods may be forbidden.

PRESERVATIVES IN MILK, CREAM, BUTTER AND MARGARINE.

The provisions of the Public Health (Milk and Cream) Regulations of 1912 are, briefly, as follows: The addition of preservative substance of any kind to milk intended for sale for human consumption is absolutely prohibited; also the sale, or offering, exposing or keeping for sale, of milk containing any preservative.

The addition of preservatives to cream which contains less than 35 per cent. of fat is prohibited, and the only preservatives which may be added to cream which contains more than 35 per cent. of fat are boric acid and borax, or mixtures of these, and hydrogen peroxide. Cream containing preservative must be described as "Preserved Cream," on a label on the vessel in which it is sold, and in all advertisements, price lists, etc., used in connection with its sale; if it contains boric acid or borax, the amount of these substances, calculated as boric acid (H_3BO_3) must be accurately stated on the label as not exceeding a certain limit.

The main reasons why preservatives should be excluded from milk are stated in the circular letter of the Local Government Board, dated July 11th, 1906, as follows: "Under the influence of these preservatives,[1] milk may be exposed without sensible injury to conditions which would otherwise render it unsaleable. It may remain sweet to taste and smell, and yet have incorporated disease germs of various kind, whereof the activity may be suspended for a time by the action of the preservative, but may be resumed again after the milk has been digested. The Committee, after hearing evidence from milk traders, concluded that the addition of preservatives to milk is not necessary for the purposes of the milk trade . . . and the Committee recommended that no preservatives should be added to milk." A further important reason for the above recommendation was the fact that milk is largely consumed by children and invalids, *i.e.*, individuals who would be especially susceptible to the harmful influence of any preservative.

The addition of preservatives to milk is prohibited in most other countries.

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[1] The evidence on which these observations were based was chiefly in connection with boric acid and formaldehyde.

With regard to cream, no definite limit has been set on the amount of boric acid which may be used for its preservation. In his report to the Local Government Board on the 'Use of Preservatives in Cream, 1909, Dr. J. M. Hamill recommended that the maximum amount of boric acid (H_3BO_3) should be 0·4 per cent. from May to October, inclusive, and 0·25 per cent. during the rest of the year; for the present, however, it is probable that prosecution under the Sale of Foods and Drugs Act would not succeed for less than 0·5 per cent. of boric acid. In the report mentioned above, it is pointed out that as cream is used to a considerable extent as food for children and invalids, individuals specially sensitive to boric acid, the declaration of the presence of this substance should be made obligatory, in order that it may be avoided by those who object to it.

In France and most of the United States of America, the use of preservatives in cream is prohibited by law; in Germany, persons selling preserved cream are liable to proceedings under the Nahrungsmittelgesetz of 1879.

As regards butter and margarine, it was recommended in the circular letter of the Local Government Board referred to above, that the only preservative allowed in these foods should be boric acid or borax, in proportions not exceeding 0·5 per cent., expressed as H_3BO_3. It is probable that for the purposes of the Sale of Foods and Drugs Act, the limit would be placed at 0·5 per cent. of boric acid; thus, a prosecution for 51 grains per pound[1] of boric acid in margarine succeeded, while a conviction for 25 grains per pound of boric acid in butter was quashed at the Quarter Sessions. It is, however, doubtful whether the use of any preservative other than boric acid (or salt) would, in itself, be considered an offence.

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[1] 35 grains per pound = 0·5 per cent.

In Germany, Holland, and some other countries, preservatives, except salt, are prohibited in butter and margarine.

Before starting on the description of the methods for the detection of preservatives in milk, cream, butter and margarine, it may be mentioned that milk is rarely treated with preservatives; formaldehyde is the preservative which has most commonly been found in milk; this and boric acid are the only preservatives which need be looked for in routine practice. On account of the importance of excluding preservatives from milk, however, methods for detecting most of the commoner preservatives in this article of food are given below.

Sodium Carbonate or Bicarbonate in Milk.—These substances have no antiseptic action, but would be added in order to neutralise the lactic acid produced in the souring of the milk (see Chapter VI.), and thus to prevent coagulation. The growth of the bacteria is not impeded.

According to Padé, the ash of 10 c.c. of genuine milk should require only 1 drop of decinormal acid for neutralisation (indicator methyl orange); if an alkali carbonate has been added, more acid will be required for neutralisation of the ash.

The estimation of added carbonate by this method may be interfered with owing to the conversion of some of the carbonate to phosphate during the incineration; in order to allow for this, it is recommended that the soluble phosphate should be determined in the ash, recalculated to sodium carbonate, and added to the amount of carbonate already found by titration.

Soxhlet and Scheibe estimate the carbon dioxide in the ash of the milk; they state that ash from genuine milk should not yield over 2 per cent. of carbon dioxide.

A test sometimes used is the rosolic acid test. To about 10 c.c. of milk are added an equal volume of 95 per cent. alcohol, and two or three drops of a solution of rosolic acid made by dissolving 0·1 gram of rosolic acid in 2·5 c.c. of alcohol and diluting to 100 c.c. with water. A rose pink coloration indicates the presence of sodium carbonate.

Milk from cows with diseased udder often has an abnormally low acidity; for the detection of udder disease, Höyberg recommends Hilger's test, which consists in adding to 5 c.c. of milk half a c.c. of a 1 per cent. solution of rosolic acid in 96 per cent. alcohol. If the reaction is normal, an orange colour is obtained; if alkaline, the mixture becomes red.

Sodium carbonate has been known to have been added to milk in the proportion of 1 part of the anhydrous salt per litre.

Detection of Boric Acid (or Borax). (a) In Milk or Cream.—Boric acid or borates may be detected by means of the characteristic reaction with turmeric. The test is very conveniently carried out in the form recommended by Bolton and Revis: About 1 c.c. of the milk is placed in a flat dish and well mixed with a drop of strong hydrochloric acid, and then with a drop of a saturated alcoholic solution of turmeric. The mixture is evaporated to dryness on the water bath; when the residue is dry, boric acid, if present, will give rise to a salmon pink colour, as little as 0·02 per cent. being detectable.

(*b*) *In Butter or Margarine.*—Boric acid may be detected by examining, as described above, the aqueous serum which collects beneath the fat when the butter or margarine is melted and allowed to stand in a cylindrical vessel at about 60° for an hour or two.

The Estimation of Boric Acid. (*a*) *In Milk or Cream.*—The most generally used method for the estimation of boric acid depends on the fact that while free boric acid does not react acid towards phenol phthalein, it forms compounds, usually looked on as condensation products, with polyhydric alcohols, such as glycerol and mannitol, which can be titrated with caustic soda solution in the presence of phenol phthalein. Under these circumstances, boric acid (H_3BO_3) behaves, in effect, as a monobasic acid.

The most convenient method of estimating boric acid in milk or cream is that of Richmond and Miller: To 10 c.c. of the milk or 10 grams of cream is added half its volume of a half per cent. solution of phenol phthalein in alcohol; decinormal caustic soda solution is run in till a pink colour appears, when the mixture is boiled and titrated back with decinormal acid till white, and then with decinormal caustic soda till very faintly pink. Glycerol is then added in amount sufficient to make up 30 per cent. of the total volume, and the titration with decinormal alkali is continued without further heating. The number of c.c. of decinormal alkali used in the last titration, less the blank value for the glycerol, multiplied by 0·0062, gives the boric acid as H_3BO_3.

(*b*) *In Butter and Margarine.*—The following method, which is based on the same principles as the preceding one, is convenient and rapid. 25 or 50 grams are melted and heated in a flask with 25 or 50 c.c., respectively, of a solution containing 0·1 per cent. of concentrated sulphuric acid and 5 per cent. of sodium sulphate. The mixture is shaken from time to time and heated in a boiling water bath until the curd is seen to have been coagulated. When the fat and water layers have separated, the latter is blown off by means of a wash bottle arrangement through a dry filter. To 20 c.c. of the filtrate is added a drop of methyl orange solution, and the liquid is neutralised by running in decinormal caustic soda, or, if preferred, a slight excess of soda may be added, and the adjustment made by running in decinormal sulphuric acid. 2 to 3 c.c. of half per cent. phenol phthalein are added together with one-third the total volume of glycerol or 1·5 gram of mannitol, and the liquid is titrated with decinormal caustic soda till a faint pink colour is obtained. The number of c.c. of soda solution used in the final titration, multiplied by 0·0355, gives the percentage of boric acid in the sample. Usually a small blank value amounting to 0·02 is found, and this may be deducted from the percentage found. The above factor takes into account the dilution owing to the water introduced with the sample, and gives results sufficiently accurate for most purposes. If desired, a factor may be calculated from the known water percentage of the actual sample, 1 c.c. of decinormal soda being equivalent to 0·0062 gram of boric acid. The remainder of the filtered liquid may be used for the estimation of salt.

Estimation of Salt in Butter and Margarine.—5 c.c. of the filtered liquid obtained in the preceding process are diluted with 20 c.c. of distilled water and titrated in the usual manner with decinormal silver nitrate solution, using potassium chromate as indicator. The number of c.c. of silver nitrate solution, multiplied by 0·13, gives the percentage of salt in the sample. This factor is obtained in the same way as the factor for boric acid given above.

Detection of Salicylic and Benzoic Acids.—These substances may be added as such, or in the form of their sodium salts. In most countries their use as preservatives for butter or margarine is not allowed, and in France they are definitely prohibited by law, in all foods.

(*a*) *In Milk.*—Girard recommends the following process for the detection of salicylic acid in milk; it may also be applied for the separation of benzoic acid: 100 c.c. of the milk are diluted with 100 c.c. of water at 60°, treated with 8 drops of acetic acid and 8 drops of a saturated solution of mercuric nitrate, shaken and filtered. The acid filtrate, which has thus been freed from fat and proteins, is extracted with ether, which will take up the salicylic or benzoic acid. Chloroform is, however, preferable as it does not take up water to the same extent as ether. The ethereal or chloroform layer is separated off, filtered through a dry filter and allowed to evaporate spontaneously in a dish; salicylic or benzoic acid, if present, will then be obtained as a white crystalline powder. For identification, the crystals may be sublimed on to a watch glass and tested for their melting point; salicylic acid melts at 155° to 156°, and benzoic acid at 121°. The following reactions may also be used for the identification of these acids: In neutral aqueous or alcoholic solution, salicylic acid gives a fine violet coloration on the addition of a drop or two of neutral ferric chloride solution, and benzoic acid gives a buff-coloured precipitate with the same reagent in neutral aqueous solution. If an aqueous solution of benzoic acid is warmed for five to ten minutes on the water bath with a few c.c. of a 0·5 per cent. solution of hydrogen peroxide, the benzoic acid is partially converted into salicylic acid, which may be recognised by the reaction just described. A delicate reaction for the detection of benzoic acid in the absence of salicylic acid is given under the next heading. (See also p. 434.)

(*b*) *In Butter and Margarine.*—The following method has been devised by Robin[1] for the detection of benzoic acid; it may also be adapted for the separation of salicylic acid: 25 grams of the melted butter or margarine are shaken in a separating funnel with 50 c.c. of a 1 per cent. solution of sodium bicarbonate, 15 c.c. of alcohol being added in order to facilitate the separation of the fat from the aqueous liquid. After shaking with a rotary motion, so as to avoid the formation of a troublesome emulsion, and allowing the layers to separate, the aqueous alcoholic liquid is run off, treated with 7 to 8 drops of sulphuric acid, heated to boiling, shaken with a little fuller's earth (in order to facilitate the separation of the proteins), and filtered through a wet filter. The cooled filtrate is shaken out with 40 c.c. of ether, and the ethereal extract, containing the benzoic or salicylic acid, is separated off and shaken with 20 c.c. of water and 5 c.c. of alcohol to remove excess of mineral acid, and then with 20 c.c. of a 1 per cent. solution of sodium bicarbonate and 5 c.c. of alcohol. The alkaline solution, which will contain the benzoic or salicylic acids as sodium salts, is run into a small dish and evaporated to dryness on the water bath. A small portion of the residue may now be tested for salicylic acid by dissolving in water and adding neutral ferric chloride to the neutral solution; if salicylic acid be absent, the bulk of the residue may be tested for benzoic acid by the following delicate reaction: Add 5 c.c. of concentrated sulphuric acid and 10 drops of fuming nitric acid, and heat carefully until white fumes are given off. Pour the resulting mixture, which should be colourless or light yellow, into 50 c.c. of water, and add sufficient concentrated ammonia solution to give a decidedly alkaline reaction. After cooling, add ammonium sulphide solution drop by drop; if benzoic acid was present in the sample, an orange yellow coloration will be produced, depending on the formation of the ammonium salt of an amido nitro benzoic acid. By this method, the presence of 0·05 per cent. of benzoic acid in the sample may easily be detected. (See also p. 434.)

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[1] Ann. de Chim. Appl., 1908, 13, 431, abs. *Analyst*, 1909, 18.

The Detection of Formaldehyde in Milk.—This preservative is generally looked on as poisonous; a further objection to its use is that it enters into chemical combination with proteins. It should be tested for in milk before the sample is too old, as it (the formaldehyde) disappears in time.

Hehner's test for formaldehyde in milk, as modified by Richmond and Bosely, is as follows: The milk is diluted with an equal bulk of water, and 94 per cent. sulphuric acid containing a trace of a ferric salt is added in such a way that it will form a layer under the milk; in the presence of formaldehyde, a violet coloration is formed at the junction of the layers; in the absence of formaldehyde, only a brownish yellow tinge will be observed. This test, which is of extreme delicacy, is only applicable to milk. If the indication is watched for, formaldehyde may be detected in the course of the carrying out of the Gerber or similar test (see p. 269). The commercial acid used contains sufficient iron, as a rule, to give the reaction.

Another method is to add to 100 c.c. of the milk 1 c.c. of dilute sulphuric acid (1 part acid to 3 of water), and to distil over about 20 c.c. Formaldehyde may be tested for in the distillate by a variety of reactions. Schiff's reagent, which consists of a solution of magenta which has been bleached by the addition of just the requisite amount of sulphurous acid, gives an intense red coloration in the presence of formaldehyde after standing for a few minutes. It is pointed out by Richmond that the distillate should be rendered faintly acid with hydrochloric acid before adding the reagent. Hehner's test for formaldehyde consists in the addition of a drop of a dilute aqueous solution of phenol, and running strong sulphuric acid down the side of the test tube. A bright crimson coloration is formed at the junction of the layers, if formaldehyde is present. Monier Williams[1] has called attention to a proprietary preservative, "mystin," consisting of sodium nitrite and formaldehyde. Nitrous acid masks the Hehner test for formaldehyde, but he points out that the addition of a little urea destroys the nitrous acid whereupon the reaction may be obtained.

In addition to the objections to the use of formaldehyde in milk, already mentioned, it may be pointed out that this preservative has been shown by Sommerfeld to have a more pronounced action on the relatively harmless organisms, *e.g.*, the lactic acid producing bacteria, than on the pathogenic and putrefactive organisms which may occur in milk. This was found to be the case when formaldehyde was added in the proportion of 1 to 10,000. It is obvious that if the activity of the harmless bacteria is impaired to a greater degree than that of the more injurious organisms, the latter will be able to develop more freely and render the milk unfit for use. (Compare Chapter VI., p. 283 *et seq.*)

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[1] *Report to Local Government Board on Public Health and Medical Subjects, New Series, No. 60, Food Reports, No. 17.*

The Detection of Hydrogen Peroxide in Milk.—Like formaldehyde, this preservative is gradually decomposed by milk, and disappears after a time. Hydrogen peroxide is decomposed into water and oxygen by a milk enzyme known as catalase, which is looked on as distinct from the enzyme peroxidase, to which the Storch reaction is due (see p. 287). The catalase test is based on the measurement of the amount of oxygen liberated from hydrogen peroxide under standard conditions, high values being obtained with milk derived from cows suffering from udder disease owing to the fact that blood corpuscles are rich in catalase. As catalase also appears to be secreted by some micro-organisms, the test only has diagnostic value when applied to fresh milk. Hinks[1] has shown that if more hydrogen peroxide is added to milk than can be decomposed by the catalase present, the excess of hydrogen peroxide may persist for a prolonged period. The hydrogen peroxide is decomposed into water and oxygen by a catalytic action, the nascent oxygen having a bactericidal action. This principle was utilised in Budde's process for sterilising milk. Hydrogen peroxide was usually added in the proportion of about 0·05 per cent.; sometimes the milk was heated with the hydrogen peroxide in closed vessels to 50° for 8 to 10 hours, after which it was supposed to be sterile. It has, however, been shown that the hydrogen peroxide does not destroy all the pathogenic organisms when used in the concentration mentioned, while if the amount necessary for complete sterilisation *i.e.*, 0·4 per cent., is added, a bitter taste is produced.

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[1] *Analyst*, 1915, 482.

Hydrogen peroxide may be detected by adding t about 10 c.c. of the milk a few drops of a freshly prepared solution of potassium iodide and starch, and the a very small quantity of dilute ferrous sulphate solution; in the presence of hydrogen peroxide, a blue coloration will be developed. The reaction is extremely delicate.

The Storch test (see p. 287) may also be used for the detection of hydrogen peroxide, omitting the addition of this reagent. It is advisable to add fresh milk free from hydrogen peroxide to the sample tested to ensure the presence of peroxidase.

The Detection of Sodium Fluoride.—The milk, cream or aqueous serum from butter or margarine is made distinctly alkaline with milk of lime, evaporated to dryness and incinerated. The ash is placed in a platinum dish, moistened with water, and 5 c.c. of concentrated sulphuric acid are added. The dish is then covered with a watch glass which has been coated on the under side with paraffin wax, the latter having been scraped off in a few places. Gentle heat is applied, care being taken not to melt the paraffin. If the exposed parts of the glass become etched in the course of about half an hour, the presence of fluoride may be inferred. In the presence of compounds of boron, the test must be modified as described under the next heading.

Monier Williams (footnote, p. 418) has proposed the following rapid test for fluorides in butter and margarine: 10 grams of the sample are melted and shaken in a separating funnel with ether and 1 or 2 c.c. of water. The aqueous layer is run off into a test tube, a few drops of hydrogen peroxide added, and 1 c.c. of a solution containing about 2 per cent. of titanium sulphate in 10 per cent. sulphuric acid. In the presence of fluoride, the orange yellow colour of the titanium solution will be partially discharged, as may be seen on comparison with a blank test. The test may also be carried out on milk, using the whey obtained by curdling the milk with a little acid and filtering.

Richmond has shown that sodium fluoride has no preserving action on milk unless added in amounts of more than 0·1 per cent.

The Detection of Fluoride in presence of Boron Compounds in Butter and Margarine.—The test for fluoride is complicated by the presence of boric acid or borax, owing to the formation of fluoboric acid, which has no etching effect on glass. As many butters and margarines contain boric acid or borax, Otto and C. W. Hehner have devised the following test: 50 grams of the sample are melted and mixed with 50 c.c. of hot water; the aqueous layer is separated from the fat in a separating funnel and made alkaline with sodium carbonate. After adding an excess of calcium chloride, the liquid is evaporated to dryness; the residue is incinerated, and the ash extracted with dilute acetic acid, transferring it to a filter and washing with the acid. The boron compounds are thus dissolved, while the calcium fluoride remains on the filter. The filter with the insoluble ash is transferred to a platinum dish, dried and incinerated. The ash is then tested for fluoride as described above.

PRESERVATIVES IN MEAT, WINE AND OTHER FOODS.

Most of the methods for the identification and estimation of preservatives given above may be applied in the

case of other foods, the method of extracting the preservative being varied to suit any particular case. Solid or semi-solid foods, such as meat or sausages, are usually comminuted and extracted with water; the filtered aqueous extract may then be acidified and extracted with chloroform, when such preservatives as benzoic and salicylic acids, or saccharin, will pass into the organic solvent, while fluorides, boric acid, etc., will remain in solution in the aqueous layer. In some cases it may be convenient to render the aqueous extract alkaline and to evaporate it to a small bulk before acidifying and extracting with ether. Liquids may be treated in the same way as the aqueous extract from solid foods. Another general method is to steam distil the material in presence of phosphoric acid, when benzoic and salicylic acids, etc., and sulphurous acid, from sulphites, will pass over with the distillate.

As examples of the methods generally in use, the detection, and in some cases also the estimation, of some of the commoner preservatives in meat and wine will be described. Besides common salt, nitre, sugar, wood smoke, vinegar and spices, the commonest preservatives used in meat and meat food products are formaldehyde, boric acid or borax, and sulphites (usually sodium or calcium bisulphites). The use of the latter preservatives in meat is forbidden by law in Germany and the United States. American packers may, however, under the direction of the foreign purchaser or his agent, add preservatives to meat and meat food products intended for export, in proportions which do not conflict with the laws of the foreign country to which they are to be exported. As has already been pointed out, the law of the United Kingdom contains no definite prohibitions for the use of preservatives in foods, except in the case of milk and cream. It may be mentioned that a conviction for 29 grains of boric acid per pound in sausages was quashed under section 6 of the Food and Drugs Act of 1875, while prosecutions have succeeded for 40 grains of boric acid per pound, and larger quantities, in similar foodstuffs. Formaldehyde is generally held to be harmful; it is used for fumigating meat intended for long transit, and is a very efficient preservative, but has a harmful effect on the digestion.

The Departmental Committee on Preservatives and Colouring Matters in Foods, 1901, recommended that the use of formaldehyde or its preparations in foods or drinks be absolutely prohibited. In a report to the Local Government Board, 1909, Dr. Buchanan recommended that meat traders and importers should consider the practicability of limiting the use of formaldehyde to the adequate disinfection of the holds in which the meat is to be conveyed, before it is introduced. In a report to the Local Government Board,[1] Dr. MacFadden points out that a considerable proportion of the samples of canned meat foods, both of British and American manufacture, examined by different analysts, contained either boron or sulphite preservatives. In reviewing he recommends that "steps should be taken to secure that specified chemical preservatives should not be used in the preparation of canned meats intended for use in this country," and that "in any schedule of prohibited preservatives, boron compounds, sulphites and preparations of sulphurous acid, benzoic acid and formalin should be included."

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[1] **Reports of Inspector of Foods, No. 6. "On Preservatives in Meat Foods Packed in Cans or Glass." 1908.**

The chief preservative used in wines, beers and other beverages, is salicylic acid. The Departmental Committee on Preservatives and Colouring Matters in Foods, 1901, recommended that this preservative should not be used in a greater proportion than 1 grain per pint in liquid food, and 1 grain per pound in solid food. Quite a number of prosecutions have been successfully maintained against salicylic acid, though the results show but little uniformity; thus cases may be cited in which prosecutions for 13 grains per pint in ginger wine failed, and 7·2 grains per pint in similar material procured conviction. A prosecution for 1·7 grains per lb. in jam failed, while 2·67 grains per lb. procured conviction. Besides salicylic and benzoic acids, beverages may be preserved with sulphites, fluorides and boric acid. Saccharin is sometimes added to wines and sweet beverages in order to reduce the amount of sugar and lessen the fermentation; it is said to conceal inferior quality, and also to have a harmful effect on digestion. Fruit juices, jams, etc., may be preserved with benzoic, salicylic, boric and formic acids.

In Germany, practically all the preservatives mentioned above (and in the case of acids, also their salts) are forbidden by law in meat and wine. Sulphurous acid and sulphites, not being considered poisonous, are not excluded from wine. They are, however, prohibited in meat, as sulphurous acid tends to restore the colour of bad meat. In France, most of the common preservatives are forbidden in wine, while the use of salicylic acid, benzoic acid, and their salts, and formaldehyde as preservatives is forbidden altogether.

Detection and Estimation of Formaldehyde in Meat.—The reaction given above for the detection of formaldehyde in milk, depending on the formation of a violet coloration in the presence of proteins, mineral acid and an oxidising agent, cannot be applied here, as meat gives a violet colour on warming with mineral acid in the absence of the aldehyde. The following method has been devised by Dr. Schryver (Report to Local Government Board on the Application of Formaldehyde to Meat, 1909) for the detection of formaldehyde, polymerised formaldehyde or formaldehyde which has entered into combination with other substances, *i.e.*, the proteins of the meat: 10 grams of the minced meat are heated for 5 minutes on a boiling water bath, with water to every 10 c.c. of which have been added 2 c.c. of a 1 per cent. solution of phenyl hydrazine hydrochloride. The quantity of liquid is varied according to the amount of formaldehyde present. In most cases where the amount of formaldehyde is 1 part in 50,000 or less, 10 c.c. of water and 2 c.c. of phenyl hydrazine solution are employed. Where the concentrations are higher, larger quantities of the liquid must be used. Thus, where the concentration of the aldehyde in the meat reaches 1 part in 5,000, 10 grams of meat are heated with 100 c.c. of water and 20 c.c. of 1 per cent. phenyl hydrazine hydrochloride solution. After heating, the liquid is cooled and filtered from the coagulum through a loose plug of cotton wool. To 12 c.c. of the filtrate are added 1 c.c. of a 5 per cent. solution of potassium ferricyanide and 4 c.c. of concentrated hydrochloric acid for each 12 c.c. of water and phenyl hydrazine reagent employed in the test. In the presence of formaldehyde, a brilliant fuschine-like colour is developed, which reaches its full intensity after a few minutes' standing, and keeps without marked deterioration for several hours. By comparison of the colour with standard solutions containing known amounts of formaldehyde, the amount of formaldehyde in the meat sample can be ascertained.

Detection and Estimation of Boric Acid (or Borax). (*a*) *In Meat.*—The following is the German official method for meat inspection: 50 grams of the comminuted meat are triturated with 50 c.c. of water, to which has been added 0·2 per cent. of concentrated hydrochloric acid (specific gravity 1·124). The mixture is allowed to stand in a beaker for half an hour, after which it is heated on a boiling water bath for half an hour, with occasional stirring, the beaker being covered with a watch glass. The warm mass is pressed in muslin, and the liquid extract poured through a moist filter. The filtrate is made faintly alkaline to phenol phthalein by the addition of decinormal sodium hydroxide solution, and evaporated to 25 c.c. 5 c.c. of the liquid thus obtained are acidified, filtered and tested with turmeric paper as follows:—

A strip of turmeric paper 8 cm. long and 1 cm. wide is wetted half its length with the acid liquid and dried on a watch glass at 60° to 70°. If no change is observed on the part which was wetted, then boric acid is absent. If a red or orange-red colour is produced, a little 2 per cent. solution of sodium carbonate (anhydrous) should be added; if a reddish brown spot is produced, which does not differ from that got with pure turmeric paper and sodium carbonate, then boric acid is absent. If, however, the sodium carbonate solution produces a blue spot, then boric acid is present. If a bluish violet coloration is produced, or the indications obtained are in any way doubtful, the flame test should be applied, the official directions for which are as follows:—

5 c.c. of the concentrated alkaline solution, obtained as described above, are evaporated to dryness and incinerated in a platinum dish. The ash is well mixed with 5 c.c. of methyl alcohol and 0·5 c.c. of concentrated sulphuric acid, and the whole is transferred to a 100 c.c. Erlenmeyer flask with the aid of a further quantity of 5 c.c. of methyl alcohol. The flask is closed with a cork, and shaken at frequent intervals during half an hour, after which all the alcohol is distilled off, heating the flask in a water bath at 80° to 85°. The distillate is introduced into a glass cylinder or test tube of about 40 c.c. capacity, about 6 cm. high, and fitted with a cork carrying two glass tubes bent at right angles, one of which passes to the bottom of the vessel, and the other just through the cork. A current of hydrogen is passed through the liquid, and ignited as it emerges from the shorter tube; if a green-edged flame is produced then boric acid is present.

Estimation of Boric Acid.—This may be carried out by evaporating a definite proportion of the concentrated alkaline liquid used for the above tests to dryness, incinerating as described under the green-flame test, dissolving the ash in water, and titrating the liquid previously neutralised to methyl orange with decinormal sodium hydroxide solution in presence of glycerol and phenol phthalein, as described on p. 413.

(*b*) *In Wine, Fruit Juices, Jams, etc.*—The following method is due to Allen and Tankard: 100 c.c. of the liquid are evaporated to dryness with 10 c.c. of a 10 per cent. solution of calcium chloride. In the case of solid or semi-solid material, such as jams, mincemeat, etc., the mass should be broken up and the calcium chloride solution well mixed with it. The residue is incinerated by first charring, then extracting the mass with 150 c.c. of water, and filtering the aqueous extract from the coal, which is burnt off by itself. The residue thus obtained is boiled with a second portion of 150 c.c. of water, allowed to stand for 12 hours and filtered cold. The filtered liquid is united with that previously obtained from the charred mass, and the boric acid in solution estimated by titration as described on p. 413. The incinerated residue may be extracted with a further 150 c.c. of water, and the filtrate thus obtained titrated for boric acid, to make sure that this constituent has been completely extracted. The qualitative tests for boric acid may be carried out as described under the preceding heading (Detection of Boric Acid in Meat) on a portion of the aqueous extract previously made alkaline, and concentrated or evaporated to dryness as the case may require.

Detection and Estimation of Sulphurous Acid or Sulphites in Meat, Wine, etc.—The following are the German official methods for the detection and estimation of sulphurous acid and sulphites in meat and wine; they may also be applied to other materials, such as jams, cider, beer, etc.:—

The qualitative test is carried out as follows with meat: 30 grams of the comminuted meat are rapidly mixed with 5 c.c. of a 25 per cent. solution of phosphoric acid in a 100 c.c. Erlenmeyer flask. The latter is closed with a cork in which a slit has been cut, so that a piece of potassium iodate and starch paper may be suspended from it. The test paper should be freshly prepared by soaking filter paper in a mixture in equal parts of 1 per cent. solutions of potassium iodate and starch, drying at a gentle heat and cutting into strips of convenient size.

A strip of this paper is suspended so that the end is about 1 cm. above the meat, and 1 cm. of the lower portion is moistened with water. If in the course of 10 minutes no blue colour appears (usually seen at the junction of the wet and dry parts of the paper), the cork is loosened and the flask is placed on the water bath, warmed, then closed with the cork and allowed to cool; if no colour is observed on the test paper after half an hour, it may be assumed that the meat is free from sulphurous acid or sulphites.

The method recommended for the estimation is as follows: 30 grams of the comminuted meat, or 100 c.c. of wine, are mixed with sufficient sodium carbonate solution to render the whole alkaline, in a 500 c.c. round-bottomed, long-necked flask, the volume of liquid being made up to about 150 c.c. After standing for 1 hour, the flask is connected up as for a steam distillation, on the one side with an inlet tube passing well below the surface of the contents, and on the other side with a Liebig condenser which is connected at the other end by means of an adapter, with a U tube having 3 bulbs (Peligot tube), which must be capable of holding 150 c.c. of liquid while gas is being passed through it. A stream of carbon dioxide, entering through the inlet tube in the flask, is passed through the whole apparatus; when all the air has been displaced, the Peligot tube is charged with 50 c.c. of a solution prepared by dissolving 7·5 grams of potassium iodide and 5 grams of iodine in 1 litre of water (pure materials being used, so that the solution is free from sulphates), and 10 c.c. of a 25 per cent. solution of phosphoric acid are added to the contents of the flask, the cork being removed and replaced as quickly as possible; the current of carbon dioxide is maintained throughout. After connecting up the apparatus as before, about half of the liquid is distilled off, still maintaining the current of carbon dioxide. All the sulphurous acid will now have been driven over into the Peligot tube, where it will be oxidised to sulphuric acid by the iodine solution, which should remain brown throughout, showing an excess of iodine to be present. The contents of the Peligot tube and rinsings are transferred to a beaker, hydrochloric acid and barium chloride are added, the mixture boiled, the barium sulphate being precipitated, collected, washed and weighed as in an ordinary sulphate determination. The barium sulphate may then be calculated to sulphur dioxide or sulphurous acid. As already mentioned, the above methods may be applied or adapted to materials other than wine or meat. Some analysts use a definite volume of iodine solution of known strength, determined by titration with standard sodium thiosulphate solution, and estimate the iodine which has been used up in the oxidation of the sulphurous acid by a second titration with thiosulphate solution. This method is more expeditious than the one given above.

Detection of Fluorides. (*a*) *In Meat.*—The German official method is as follows: 25 grams of the minced meat are thoroughly mixed with an excess of milk of lime in a platinum dish, dried and incinerated. The residue is treated with 3 drops of water and 1 drop of concentrated sulphuric acid, the etching test for hydrofluoric acid being applied in the usual way, as described on p. 420.

(*b*) *In Wine.*—Vandam recommends the following method: To 100 c.c. of the sample in a measuring cylinder are added 0·5 to 1 c.c. of a 20 per cent. sodium sulphate solution and 10 c.c. of a 10 per cent. barium acetate solution; after shaking well, the mixture is allowed to stand overnight and the clear liquid syphoned off. The sediment is shaken up with 100 c.c. of hot water and allowed to settle; after removing the clear liquor, the process is repeated with a further quantity of 50 c.c. of hot water. The washed sediment, containing the insoluble barium fluoride, is transferred to a double filter and, when dry, incinerated. The ash is moistened with water, treated with 5 c.c. of concentrated sulphuric acid, and the etching test applied as described on p. 420. It should be noted that many wines contain small traces of fluorides as a natural constituent.

(*c*) *In Beer.*—The following method is given in Allen's "Commercial Organic Analysis," Vol. I., 1909 ed.: 100 c.c. of the sample are made slightly alkaline with ammonium carbonate, boiled, treated with 2 to 3 c.c. of a 10 per cent. calcium chloride solution and boiled again for 5 minutes. The precipitate is filtered off, washed, dried and tested for fluoride in the usual way.

Detection and Estimation of Chlorides and Nitrates in Meat.—The following method, partly due to Given, is described by Leffmann and Beam in their "Food Analysis": It will be necessary first to determine the chlorides present, as these interfere with the nitrate determination; this may be done by titrating the solution obtained by extracting 1 gram of the minced meat with 200 c.c. of water, with decinormal silver nitrate solution, using potassium chromate as indicator.

For the determination of nitrates, 1 gram of the sample is placed in a 100 c.c. flask, 50 c.c. of water are added, and the mixture warmed in hot water for 20 minutes, with occasional shaking. For each 1 per cent.

of sodium chloride found to be present, 3 c.c. of a saturated solution of silver sulphate are added, then 10 c.c. of basic lead acetate, and 5 c.c. of alumina cream (see p. 392), shaking after each addition. The liquid is made up to 100 c.c., shaken, filtered through a dry fluted filter, the filtrate being returned till clear. 20 c.c. of the filtrate are evaporated to dryness on the water bath in a porcelain dish, and the residue is mixed with 1 c.c. of phenol disulphonic acid, the preparation of which is described below; without applying heat, the acid is stirred over the whole residue; the mixture is completely transferred to a Nessler tube by rinsing with water, and the solution thus obtained made alkaline with ammonia or soda. By the interaction of the nitrate, the phenol disulphonic acid and the alkali, an alkaline picrate is formed, the depth in colour due to the latter being proportional to the amount of nitrate present in the sample. The determination may therefore be made by comparison with a solution which has been similarly prepared by evaporating to dryness on the water bath a known volume, say, 1 c.c., of a standard solution of potassium nitrate, containing 0·001 gram of the salt per cubic centimetre, treating with phenol disulphonic acid, transferring to a Nessler tube by means of water and rendering alkaline as before. The two picrate solutions are made up to the same volume and compared. If the difference in the depth of colour is not great, some of the deeper coloured solution may be poured off till the tints observed in the two tubes when placed side by side on a white surface are sensibly equal. The relative depths of the two layers of liquid may then be taken as a basis for calculation. If, on the other hand, the difference in tints is very marked, another solution for comparison must be made from a greater or smaller quantity of potassium nitrate.

The phenol disulphonic acid is prepared as follows: 37 grams of pure sulphuric acid and 3 grams of pure phenol are heated for 6 hours in a flask immersed in boiling water. The resulting reagent may crystallise on cooling, but can easily be liquefied on gentle warming.

Detection and Estimation of Salicylic and Benzoic Acids.—The following method is recommended by Harry and Mummery for the detection and estimation of salicylic acid in wine and beer; it has the advantage of eliminating tannins which may mask the reaction of salicylic acid with ferric salts, and also substances which tend to give rise to emulsions on extracting with immiscible solvents. It may equally well be applied to solid or semi-solid foods, in which case it will be necessary to make either an aqueous solution or a slightly alkaline aqueous extract. As far as the actual extraction of the preservative from the sample is concerned, the method is also applicable to benzoic acid.

100 c.c. of the sample (or aqueous solution or extract) are made alkaline with 5 c.c. of normal sodium hydroxide solution, and the alcohol (if any) is driven off at a temperature just below the boiling point. The following operations, up to the ether extraction, have for their object the removal of tannins and pectinous or albuminous matter from the aqueous solution of the preservative. 5 c.c. of normal hydrochloric acid are added, and then 20 c.c. of basic lead acetate solution; the mixture is made alkaline with about 20 c.c. of normal sodium hydroxide solution, and made up to 200 c.c. with water. The tannins are precipitated while the lead salicylate (or benzoate) is soluble in the alkaline solution. After

mixing, heating in boiling water and cooling, the liquid is filtered through a dry filter; 100 c.c. of the filtrate are acidified with hydrochloric acid, which will precipitate albuminous matter together with lead chloride, besides liberating the salicylic (or benzoic) acid. The filtrate and washings from the last precipitation are extracted three times with ether, the ethereal extracts united and evaporated to dryness.

If, however, the salicylic acid is to be estimated colorimetrically by the method described below, it is better not to evaporate the ethereal solution, as loss of salicylic acid may occur through volatilisation with the water contained in the ether. A better plan will be to extract the ether solution with 1 per cent. sodium bicarbonate solution, and to work with the neutralised solution of sodium salicylate. If it is desired to isolate the acid for determination of the melting point, another portion may be employed. Chloroform is better than ether as an extracting medium (see p. 415).

The identification of salicylic or benzoic acids may be carried out by the methods already described (see p. 415 and below). For the estimation of salicylic acid, Harry and Mummery recommend the following method: The acid is dissolved in a small quantity of dilute alcohol and made up to 100 c.c. in a Nessler tube. The colour produced with ferric chloride solution is then compared with the colour produced on adding an equal amount of ferric chloride to solutions of the same volume in Nessler tubes containing known amounts of salicylic acid.

In the presence of both benzoic and salicylic acids, van der Laan and Tydens recommend the following method for their separation: After estimating the salicylic acid colorimetrically (see above) in a portion of the aqueous solution, the rest of the solution is extracted with ether (or chloroform, see above), the acids obtained by evaporation of the ethereal extract are dissolved in 10 to 20 c.c. of quarter-normal caustic alkali solution, and a slight excess of a 5 per cent. potassium permanganate solution is added. After gentle heating by means of a small flame, the excess of permanganate and the separated manganic hydroxide are reduced by adding a saturated solution of sulphur dioxide. The salicylic acid will be destroyed by the oxidising agent, while the benzoic acid will remain unaffected. The acidified solution may then be extracted with ether, and the benzoic acid thus obtained identified, and estimated by titration. For the method of separating salicylic and benzoic acids from saccharine, which may mask the reactions for the identification of the former, see below.

Detection and Estimation of Saccharine in Beverages.—Saccharine, or ortho-benzoyl-sulphone-imide or its sodium salt, is not a preservative in the true sense of the word; it is added to beverages in place of sugar, so as to reduce fermentation.

The imide is removed on extracting the acidified material with ether, and may be detected by the sweet taste of the residue obtained on evaporation of the ether. If present together with benzoic and salicylic acids, it will generally accompany these if they are extracted from the material by means of organic solvents. Separation may be accomplished by acidifying 200 grams of the sample with 5 c.c. of a 20 per cent. phosphoric acid solution, and distilling almost to dryness; the acids will be found in the distillate, from which they may be obtained by acidifying and extracting with ether, while the saccharine will remain in the flask, and may be obtained by extracting the contents with water and extracting the acid solution thus obtained with ether in the usual way.

Allen's process for the estimation of saccharine in beer is as follows: The beer is concentrated to one-third of its original bulk, and if not acid, it is rendered so by the addition of a little pure phosphoric acid. The liquid is extracted with ether, the latter evaporated, and the residue mixed with an excess of anhydrous sodium carbonate and a little potassium nitrate, and ignited till all organic matter has been burnt off. A determination of sulphate in the residue is made by dissolving in water, acidifying with hydrochloric acid, adding barium chloride solution, etc., as usual. The factor for calculating the barium sulphate to saccharine is 0·785. Care should be taken that the reagents employed are free from sulphur compounds.

Detection and Estimation of Formic Acid.—This preservative is chiefly used for fruit juices and preserves. In honey it is present as a natural constituent in quantities up to 0·21 per cent.

According to Croner and Seligmann, formic acid is separated from the sample by mixing 100 grams with 400 c.c. of water, acidifying with phosphoric acid and distilling in steam. Fincke (see below) acidifies with tartaric acid, which is preferable as it is less liable to decompose the organic matter than mineral acid. 500 c.c. of distillate are collected, sufficient caustic soda solution being placed in the receiver to keep the whole alkaline. The alkaline distillate is evaporated to 10 c.c., treated with an excess of baryta solution and filtered; the excess of baryta is then precipitated as sulphate by the addition of sulphuric acid, and the liquid again filtered. By this treatment, acids other than formic acid, which might mask the reactions for the latter are removed. The filtrate is boiled with mercuric chloride solution, when, in the presence of formic acid, a precipitate of mercurous chloride will be produced. According to Smith, formic acid may be identified by adding to the acid steam distillate a slight excess of ammonia above that required for neutralisation, evaporating to a small bulk and adding a few drops of neutral ferric chloride solution; in the presence of formic or acetic acids, a red coloration will be produced; on shaking with 96 per cent. alcohol, a precipitate will be produced in the presence of formic acid, but not in the presence of acetic acid only. An excess of acetic acid interferes with the reaction; in such a case, the acid steam distillate is partially neutralised with about 5 c.c. of normal soda solution, and concentrated to 15 c.c.; most of the formic acid will remain combined with the alkali, while most of the acetic acid will be evaporated off.

H. Fincke[1] recommends the following method for the estimation of formic acid: To the neutral or faintly acid steam distillate containing all the formic acid, obtained as described above (see also below), are added 3 to 5 grams of sodium acetate and at least 15 times as much mercuric chloride (in solution), by weight, as there is formic acid present. The mixture is heated for 2 hours in a flask fitted by means of a rubber stopper with a tube condenser, immersed in boiling water which reaches to the level of the liquid in the flask. The mercuric chloride solution is prepared by dissolving

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[1] Zeitschr. für Nahr. u. Genussmittel, 1911, 21, 1 and 1911, 22, 88. Abs. *Analyst*, 1911, 103 and 496.

100 grams of mercuric chloride and 30 grams of sodium chloride in 100 c.c. of water. The precipitated mercurous chloride is filtered off on a Gooch crucible, washed with hot water, alcohol and ether, dried and weighed. The factor for converting mercurous chloride into formic acid is 0·0977.

As regards the steam distillation, it will be necessary to distil at least 500 c.c., in order to bring practically all the formic acid over. As the estimation of the formic acid depends on the reducing action of the steam distillate, errors may be introduced through the presence of volatile aldehydes, which may exist as such in the sample or be produced from tartaric and other acids. To obviate this, the steam may be led through two flasks containing a suspension of calcium carbonate, before it enters the condenser; if the flasks are kept heated, the aldehydes will pass on with the steam, while the formic acid is retained in the flasks as calcium formate. After filtering off the calcium carbonate and washing with water, the formic acid is estimated in the filtrate as described above. Care should be taken to avoid spray being carried over in the distillation, as reducing sugars may be present in the sample, and these would vitiate the result by reducing some of the mercuric chloride. If sulphurous acid is present, the concentrated neutral distillate, or the filtrate from the calcium carbonate, is treated with about 5 c.c. of quarter-normal caustic soda solution and 5 c.c. of concentrated hydrogen peroxide solution, and left for 4 hours; the sulphite will then be oxidised to sulphate. A little freshly precipitated mercuric oxide, made into a paste with water, is then added to destroy the excess of hydrogen peroxide, and after half an hour the liquid is filtered, and the formic acid estimated in the filtrate as described above. In the presence of salicylic acid, common salt should be dissolved in the distillate; this will prevent the precipitation of mercury salicylate.

Other Preservatives.—The detection and estimation of most of the commonly occurring preservatives have been described. One or two methods for detecting a few other substances which may be used as preservatives are briefly outlined below:—

For the detection of β naphthol and other similar substances the American Association of Official Agricultural Chemists recommend that 200 grams of the acidified sample be distilled in steam, and the first 200 c.c. of the distillate extracted with 20 c.c. of chloroform; on separating the latter, adding caustic potash and heating almost to boiling for a few minutes, colour changes will occur as follows: in the presence of phenol, light red to brown, to yellow, to colourless. In the presence of salol, light red, and in the presence of β naphthol, deep blue to green, to brown.

PART II.—ARTIFICIAL COLOURING MATTERS.

INTRODUCTORY.

The colouring matters used in foods may be classed under three headings: coal-tar dyes, naturally occurring organic colours, and metallic or inorganic colours. The most frequently used are the coal-tar dyes, the majority of which are generally held to be harmless, especially in the small amounts in which they are used, provided, of course, that they are pure, and free from arsenic or lead. The great majority of the vegetable colouring matters are also harmless, but the metallic colouring matters, such as chromates, copper salts, etc., are mostly injurious, even in small amounts. The vegetable colouring matters, such as cochineal, anatto, turmeric and saffron, have largely been superseded by the coal-tar colours, while mineral colours, with the exception, perhaps, of copper sulphate, are of comparatively rare occurrence in foods. Among the few poisonous organic colours may be mentioned gamboge and picric acid.

The law of the United Kingdom does not definitely forbid the use of colouring matters in any food, or limit the amounts in which they may be used; as in the case of preservatives the addition of any objectionable colouring matter would, in all probability, be dealt with under section 6 of the Sale of Foods and Drugs Act of 1875 (see p. 407). Even when the colouring matter itself is harmless, its presence would be objectionable if it had been added in order to conceal the inferior quality of the food, as might be done, for example, in the case of meat, milk or wine.

The Departmental Committee on Preservatives and Colouring Matters in Foods, 1901, recommended that the use of colouring matters of any kind in milk offered for sale in the United Kingdom should be considered an offence under the Sale of Foods and Drugs Act. Further, that the use of copper salts in the so-called greening of preserved foods should be prohibited. These recommendations have, however, not resulted in any legislation on the subject except the Ministry of Food Order mentioned on p. 408.

The use of copper salts in foods is prohibited in Germany and Austria-Hungary. In the United States certain specified colouring matters only are allowed in foods, pending further enquiry. Copper salts are prohibited in the colouring of vegetables, particularly peas and beans.

The identification of organic colouring matters, especially coal-tar dyes, in foods, may often be a difficult task, requiring considerable experience. In many cases the full identification of the colouring matter will not be necessary; it will be sufficient to determine its nature, and to be able to say whether it is harmless or injurious. By the methods given below, the general nature of the colouring matter present may be determined; the methods for the detection of some of the commoner vegetable colouring matters are also given.

Dyeing Method for the Detection of Coal-tar Colours.—The method described is recommended by Thresh and Porter ("Preservatives in Food and Food Examination") for the detection of both acid and basic coal-tar dyes, being based on the method of Sostegni and Carpenteri for the detection of acid dyes. It may generally be employed for the detection of coal-tar dyes in meat, wine, confectionery, milk, fruit juices and extracts, etc.

The majority of the dyes found in foods are of an acid nature, only few basic colours being met with. If clean white wool is immersed in an acid solution of an acid dye, or an alkaline solution of a basic dye, it will take up the colour in both cases, forming insoluble compounds with dyes of both classes, presumably owing to its containing both basic and acid constituents. In addition to coal-tar colours, the wool will take up some vegetable colours, such as cochineal or logwood; if, however, the acid dye, for example, be dissolved from the wool by means of an alkaline solution, then, on acidification of the latter, a second piece of wool may be dyed from it if a coal-tar colour be present, but not if the first dyeing was due to a vegetable colour. The same holds good, *mutatis mutandis*, as regards basic dyes.

In the first place, it will be necessary to prepare a clear solution or extract of the material containing the colour. Solid material should be broken up by passing through a sausage grinder, when the colour may be extracted by warming with water or 80 per cent. alcohol. The filtered solution of the colour is divided into two portions of 50 to 100 c.c. each, the one of which is rendered faintly alkaline with ammonia, and the other distinctly acid with hydrochloric acid. Into each of these solutions is put about a foot of white worsted which has previously been boiled in a very dilute solution of caustic soda in distilled water, and washed till free from alkali. The solutions are kept at the boiling point for an hour, or less if the wool in one of them is distinctly dyed. The dyed wool is removed, pressed between sheets of filter paper, and washed by immersing in two successive portions of 20 c.c. of boiling water.

The wool, if dyed from the acid solution, is then immersed in about 20 c.c. of a boiling solution of dilute ammonia containing 10 per cent. by volume of the concentrated ammonia of specific gravity 0·880, or, if dyed from the alkaline solution, in the same quantity of boiling 5 per cent. acetic acid. The wools are removed, and the alkaline liquid made acid by the addition of acetic acid, and the acid liquid made alkaline by ammonia. A fresh piece of white, grease-free worsted, about 2 to 3 inches long, is placed in each solution; after heating for half an hour on a boiling-water bath, the wools are removed and washed in distilled water; if a coal-tar dye was present in the original solution, one of the samples should be brilliantly dyed; if the dye present is basic, the most distinct dyeing will take place from the alkaline solutions; if acid, from the acid solutions. In presence of vegetable colours, the second piece of worsted will acquire, at most, a dirty appearance.

For identification, the colour may be removed from the wool used in a first dyeing, as described above, either by means of acid or alkali, and the tests applied to the solution. The identification of coal-tar colours may be a difficult task, requiring some special experience; the subject is fully dealt with in Vol. V. of Allen's "Commercial Organic Analysis," where several recognised schemes are given; the other works mentioned at the end of this chapter may also be consulted.

Colouring Matters in Milk.—The artificial colouring of milk has been extensively practised owing to the erroneous popular notion that the "richer" the colour of milk the richer will it be in cream. As a matter of fact, during the summer, when the milk is naturally more yellow and yields a more highly coloured butter than in winter, the average fat percentage is usually lower than in winter (see p. 248). As mentioned above, the addition of artificial colouring matters to milk was recently prohibited in the United Kingdom by the Ministry of Food; in all probability the prohibition will remain in force indefinitely.

The colours which have been used most are anatto and azo dyes, such as methyl orange, amino azobenzene, chrysoidine, etc. These colours are generally destroyed by the action of the micro-organisms in milk (cf. the reductase test, p. 288), so that a negative result may be obtained with a coloured milk which has been kept for a few days. Anatto is more permanent than the coal-tar dyes. The colours are not restored on the addition of hydrogen peroxide or shaking with air, as is the case with methylene blue.

Anatto is detected by making the milk alkaline with sodium bicarbonate and soaking a strip of filter paper in it overnight ; a brown stain is formed on the paper, which is turned pink on moistening with stannous chloride solution.

Azo colours may be indicated by the production of a pink colour on acidifying the milk with hydrochloric acid. The following general method recommended by Richmond is more reliable. The colour is found either in the fat or in the aqueous portion : At least 60 c.c. of milk are made just alkaline to delicate litmus paper by the addition of dilute soda or strontia solution, and evaporated to a thin paste on the water bath. The paste is thoroughly extracted with ether, which will remove the fat. The ether is evaporated off, and the fat shaken with warm water. The water is separated and evaporated to dryness in a porcelain dish ; a coloured residue will be due to added colour.

The fat-free residue is extracted with absolute alcohol, which is filtered and evaporated to dryness in a porcelain dish. A coloured residue will be due to added colour.

Colouring Matter in Butter and Margarine.—As in milk, practically the only vegetable colouring matter which need be considered is anatto, which, however, has been largely displaced by certain fat-soluble azo colours. Butter only has a natural strong yellow colour during the summer ; at other times, the colour may be increased artificially. The amounts of azo colours added to butter or margarine are extremely minute, so that identification is hardly a practicable proposition. It is only possible to arrive at a rough classification by means of certain colour reactions. Owing to the weakly acidic or basic characters of many azo colours, these can often only be extracted from the fat with difficulty by means of aqueous alkali or acid.

Anatto is extracted from the fat by melting at a low temperature, mixing with an equal volume of petroleum ether and shaking with dilute caustic soda solution. The yellow aqueous extract may be tested for anatto in the same way as described in the preceding section.

If a little of the sample is melted and shaken with 10 per cent. sulphuric acid, some azo colours will produce a pink coloration in the acid layer. A more general method for detecting added colours is to dissolve a little of the clear fat in glacial acetic acid by warming, and to add 2 or 3 c.c. of 10 per cent. sulphuric acid; the lower layer will be coloured yellow, orange or green by azo colours.

Colouring Matters in Meat.—Meat is generally coloured with coal-tar dyes, such as fuschine, eosin and benzopurpurin. These may be recognised by extracting with alcohol, and applying the dyeing test to the extract as described above. Carmine and cochineal have also been used for colouring meat.

The following method, due to Klinger and Bujard, and modified by Bremer, may be used for the detection of carmine: 20 grams of the minced meat are heated on the water bath for several hours with a mixture of equal parts of glycerol and water which has been slightly acidulated with tartaric acid; the liquid is separated by straining through muslin, and when cold, filtered in order to separate it from fat and suspended matter. On adding alum solution and then ammonia, the precipitated aluminium hydroxide will carry the colour with it, as a lake; the latter is filtered off, washed with water, dissolved in a small quantity of tartaric acid solution, and examined in the spectroscope; if carmine is present, the solution will show absorption bands at *b* and E, and another close to D, in the solar spectrum.

Colouring Matters in Wine.—Coal-tar dyes will be detected by the dyeing test, described above; in addition to these, such materials as elderberry, logwood and cochineal are said to be in use for colouring wine. The subject is fully treated of in some of the other works mentioned at the end of this chapter. The tests used at the Municipal Laboratory of Paris for detecting artificial colouring matters in wine are given in Wynter Blyth's work.

Detection and Estimation of Metallic Colouring Matters.—The commonest metallic colouring matter used in foods is, perhaps, copper sulphate, which is added to preserved peas, spinach, etc., to impart a bright green colour and, at the same time, to harden the integument.

A general method for the detection of compounds of copper, arsenic, lead, chromium, zinc, etc., all of which are poisonous, is as follows[1]: 100 grams or more of the material is mixed in a flask with a tenth of its weight of potassium chlorate and sufficient water to produce a thin paste in the next operation. Hydrochloric acid gas is passed through the mixture, the escaping gas being led through water to arrest possible traces of arsenic chloride. When yellow vapours are seen above the liquid, the current of gas is stopped; the process of destruction will then proceed spontaneously till a straw yellow liquid is obtained. Any insoluble matter which may be present is usually free from poisonous metals, but should be examined as well as the solution by the usual methods of inorganic analysis.

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[1] See also Lander, "The Detection of Poisonous Metals," *Analyst*, 1908, 45.

Arsenic, if present, will usually have been introduced as an impurity with organic or mineral colouring matters, or other materials used in the manufacture or preparation of the food, and is, therefore, only likely to be present in very small amounts ; it must accordingly be tested for in the original material by special methods, such as the Marsh or Gutzeit test ; methods for the detection of arsenic in foods will be found fully described in some of the works mentioned at the end of this chapter.

Ferric oxide is said to be added to cocoa, in order to improve its colour, and as an adulterant ; it may be detected and estimated on the same lines as described above for other mineral additions to foods.

REFERENCES.

"Preservatives in Food and Food Analysis," by Thresh and Porter. 1906. J. & A. Churchill, London.

"Foods : Their Composition and Analysis," by A. and M. Wynter Blyth. 1909. Chas. Griffin, London.

"Foods and their Adulteration," by Harvey Wiley. 1910. J. & A. Churchill, London.

"Select Methods in Food Analysis," by Leffmann and Beam. 1905. Rebman, Ltd., London.

"Food Inspection and Analysis," by Leach. 1914. Wiley & Sons, New York.

"The Sale of Foods and Drugs Act," by J. W. Bell. 1914. Butterworth & Co., London.

"Foods and Drugs," by E. Parry. 1911. Scott & Greenwood, London.

"The Identification of Pure Organic Compounds," Vol. III. (Colours), by S. P. Mulliken. Vol. 3. 1911. Wiley & Sons, New York.

Allen's "Commercial Organic Analysis," Vol. V. (Colours). 1910. J. & A. Churchill, London.

Reports to the Local Government Board on Public Health and Medical Subjects, New Series, No. 12, Food Report, No. 12: Dr. G. S. Buchanan and Dr. S. B. Schryver on the "Application of Formaldehyde to Meat."

Reports to the Local Government Board on Public Health and Medical Subjects, New Series, No. 13, Food Report, No. 10: Dr. J. M. Hamill on the "Use of Preservatives in Cream." Wyman & Sons, London.

Local Government Board Reports of Inspector of Foods, No. 6: "On Preservatives in Meat Foods packed in Cans or Glass." Wyman & Sons, London.

Statutory Rules and Orders, 1912, No. 1152: The Public Health (Milk and Cream) Regulations.

Villavecchia's Work, Vol. II. See end of Chapter VII.

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